Author:
Turpin-Jelfs, Thomas C

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Impacts of environmental change on soil nitrogen in emerging and degrading arid ecosystems

Impacts of environmental change on soil nitrogen in emerging and degrading arid ecosystems

Thomas Turpin-Jelfs

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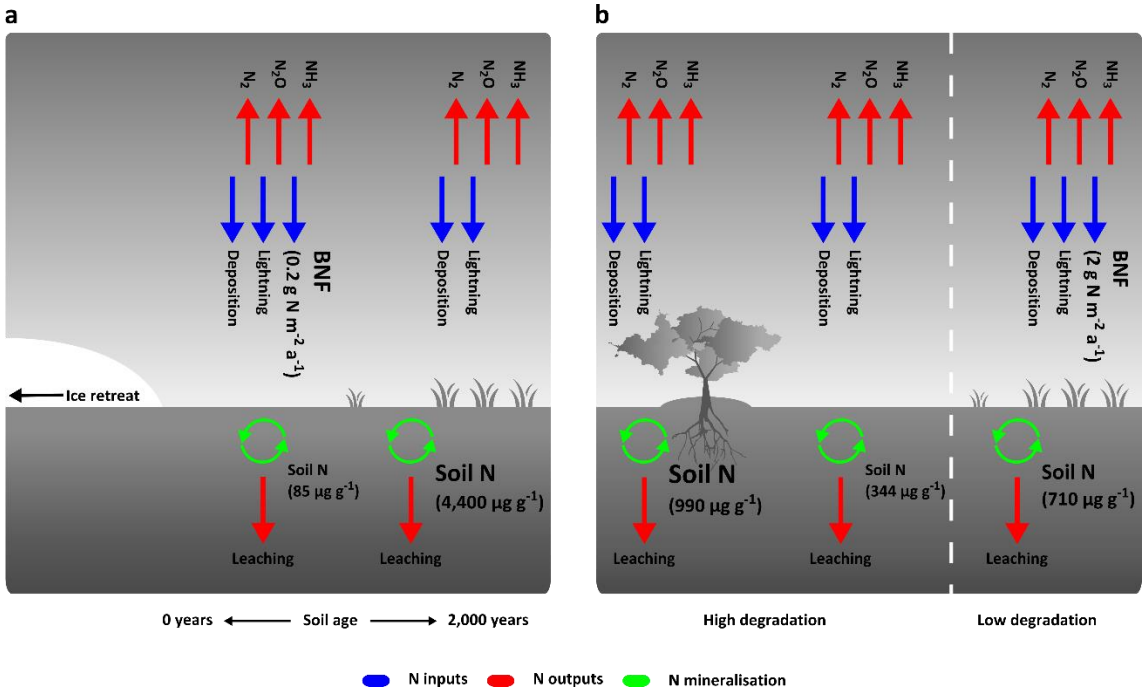
Summary

Changes in climate and land use over the past ca. 150 years have led to environmental changes associated with primary succession and land degradation in water-limited ecosystems. As a microbiologically mediated process, the cycling of nitrogen, the nutrient most limiting to primary production in water-limited ecosystems, may be perturbed by changes in external environmental parameters. Yet an understanding of how soil nitrogen responds to environmental changes between trajectories of succession and degradation in water-limited ecosystems is missing. From this research, it was determined that whilst the spatial distribution of soil nitrogen is generally influenced by surface cover, major differences arise between trajectories of succession and degradation. In a water-limited glacier forefield, total nitrogen increases with primary succession from ~85 µg g^{-1} in recently exposed soils to ~4,400 µg g^{-1} in 2,000-year-old soils, which suggests such ecosystems will become more productive over time. Conversely, the redistribution of total nitrogen (~85 g m^{-2}) from intershrub areas to soils beneath invasive shrub canopies in a water-limited grassland expedites grassland-to-shrubland transitions resulting in reduced pastoral productivity. Despite these differences, >98 % of nitrogen in these systems is organically bound. As this is indicative of nitrogen limitation, the capacity for biological nitrogen fixation should represent a selective advantage. Using the acetylene reduction assay technique, potential rates of asymbiotic biological nitrogen fixation were found to be largely modulated by inorganic nitrogen-to-bioavailable phosphorus ratios and decline from ~0.2 g N m^{-2} a^{-1} in a glacier forefield and ~2 g N m^{-2} a^{-1} in a shrub-encroached grassland with the accumulation of total nitrogen. The large fraction of organically bound nitrogen suggests the accumulation of organic matter is a significant driver of microbial activity in water-limited ecosystems. When considering the global extent of water-limited environments, changes in soil nitrogen pools within these ecosystems have implications for the global nitrogen cycle.



Conceptual model illustrating the inputs (wet and dry deposition, and fixation by lightning and biological processes), transformations (mineralisation) and outputs (emissions of dinitrogen (N_2), nitrous oxide (N_2O) and ammonia (NH_3)) of nitrogen (N) in expanding glacier forefields (a) and grasslands undergoing shrub encroachment (b), for which the distribution of soil N pools, as well as inputs of N by biological N fixation (BNF) were quantified as part of this study. Absent N inputs were determined to be below the limits of detection.

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I would like to express my heartfelt gratitude to Dr Katerina Michaelides and Prof Alexandre Anesio for their unwavering support, patience and guidance during the undertaking of this research project. I was continually inspired by their creativity and overall enthusiasm towards research. As such, it was an absolute pleasure to collaborate with them during my time at the University of Bristol.

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Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Glossary

ATP	Adenosine triphosphate
Bioavailable phosphorus	sum of loosely-sorbed, and iron- and aluminium-bound phosphorus
BNF	Biological nitrogen fixation
BSh	Hot arid steppe Köppen-Geiger climate class (see section 1.1.4)
BSk	Cold arid steppe Köppen-Geiger climate class (see section 1.1.4)
BWh	Hot arid desert Köppen-Geiger climate class (see section 1.1.4)
BWk	Cold arid desert Köppen-Geiger climate class (see section 1.1.4)
Coarse fraction	All soil particles that are >2,000 μm
C:N	Soil organic carbon-to-total nitrogen
DMC	Dry matter content
DT	Dunn's test of multiple comparisons
EF	Polar frost Köppen-Geiger climate class (see section 1.1.4)
ET	Polar tundra Köppen-Geiger climate class (see section 1.1.4)
Fine earth fraction	All soil particles that are ≤2,000 μm
GC	Gas chromatograph
Inorganic nitrogen	Sum of ammonium as nitrogen and nitrate as nitrogen
K-G	Köppen-Geiger (see section 1.1.4)
KW	Kruskal-Wallis *H* test
MAP	Mean annual precipitation
MAT	Mean annual temperature
MW	Mann-Whitney *U* test
N:P	Inorganic nitrogen-to-bioavailable phosphorus
PET	Potential evapotranspiration

PLFA	Phospholipid fatty acid
SOC	Soil organic carbon
SRER	Santa Rita Experimental Range (AZ, US; see section 4.3.1)
WHC	Water holding capacity

1 General introduction

1.1 Introduction

1.1.1 Nitrogen in the environment

Anthropogenic activities associated with fossil fuel combustion and land-use change are perturbing Earth's climate system by increasing the rates at which greenhouse gases accumulate in the atmosphere (Moura Costa and Wilson, 2000). Currently, it is projected that continued emissions of carbon dioxide (CO_2), the most significant radiatively active gas contributing to climate warming (Montzka *et al.*, 2011), will cause mean global surface temperatures to rise by 2.6-4.8 °C by 2100 (Collins *et al.*, 2013). However, these projections are subject to uncertainties regarding the response of terrestrial biomass production to elevated concentrations of atmospheric CO_2 (Reich *et al.*, 2006). This is because whilst CO_2 enhancement may stimulate the accumulation of plant biomass for the sequestration of anthropogenic CO_2 emissions (Norby *et al.*, 2010), primary productivity is often constrained over physiological timescales by the availability of nitrogen (N), a fundamental constituent of many life-critical biomolecules (Butterbach-Bahl *et al.*, 2011). Thus, improving understanding of the controls on N availability has implications for the global carbon (C) cycle, as well as the responses of ecosystems to climate-induced warming driven by rising concentrations of CO_2 in the atmosphere (Vitousek *et al.*, 1997a).

Whilst major fluxes of C are associated with net primary productivity, the cycling of N (Figure 1.1) is largely mediated by microbiological processes in the absence of anthropogenic influence (Butterbach-Bahl *et al.*, 2011). Globally, inputs of reactive N, which may be defined as N that is combined with the elements C, hydrogen (H) or oxygen (O), to unmanaged terrestrial ecosystems are derived from the fixation of atmospheric dinitrogen (N_2) by diazotrophic microorganisms in soil (90-130 Tg N a^{-1}), and to a lesser extent, lightning (3-5 Tg N a^{-1}; Galloway, 1998). Biologically fixed N is subsequently incorporated into the organic N pool (Knowles, 2009), which typically comprises >90 % of total N in soil and is an important constituent of soil organic matter (Stevenson, 1982). Microorganisms may then convert N contained within the organic phase to inorganic forms by mineralisation and nitrification processes, for which ammonium (NH_4^+) and nitrate (NO_3^-), in addition to soluble forms of organic N, are available to plant and microbial biomass (Vitousek *et al.*, 2002b). These inorganic forms of N are highly mobile in soil and are susceptible to losses by leaching along hydrological pathways, as ammonia (NH_3) through volatilsation and biomass burning, or as N_2 and nitrous oxide (N_2O), another radiatively active gas, through denitrification processes (Galloway, 1998).

The availability and retention of N in terrestrial systems is influenced by climatic and edaphic conditions, and varies by ecosystem type (Butterbach-Bahl *et al.*, 2011). Consequently, environmental

changes arising from variations in climate and land use have the potential to affect the capacity of ecosystems to store and cycle N (Delgado-Baquerizo *et al.*, 2013). Of particular concern are drylands, which are considered ecologically marginal and sensitive to increasing climatic and anthropogenic pressures (Reynolds *et al.*, 2007a). Yet the effects of environmental changes on the availability and storage of N in dryland ecosystems are poorly resolved (Browning *et al.*, 2008).

1.1.2 Nitrogen and arid ecosystems

Often estimated as comprising ~41 % of the continental surface (e.g., Ravi *et al.*, 2010a; Reynolds *et al.*, 2007b), drylands or arid ecosystems, terms used synonymously here, constitute the largest terrestrial biome (by area) on Earth (Delgado-Baquerizo *et al.*, 2013). Due to a limited supply of water and nutrients, arid ecosystems are typically characterised by low annual net primary productivity and standing biomass (Noy-Meir, 1973). After water, the supply of N has been determined to be the most significant limiting factor of primary production in arid ecosystems over physiological timescales (Gebauer and Ehleringer, 2000). Consequently, identifying the main input pathways of N to the soil system, as well as the processes controlling the subsequent cycling of N, is critical to understanding ecosystem structure and function in dryland environments (Schmidt *et al.*, 2011). Further, when the extent of drylands is considered, knowledge of these processes has implications for improving current understanding of the global N cycle (Evans and Ehleringer, 1993).

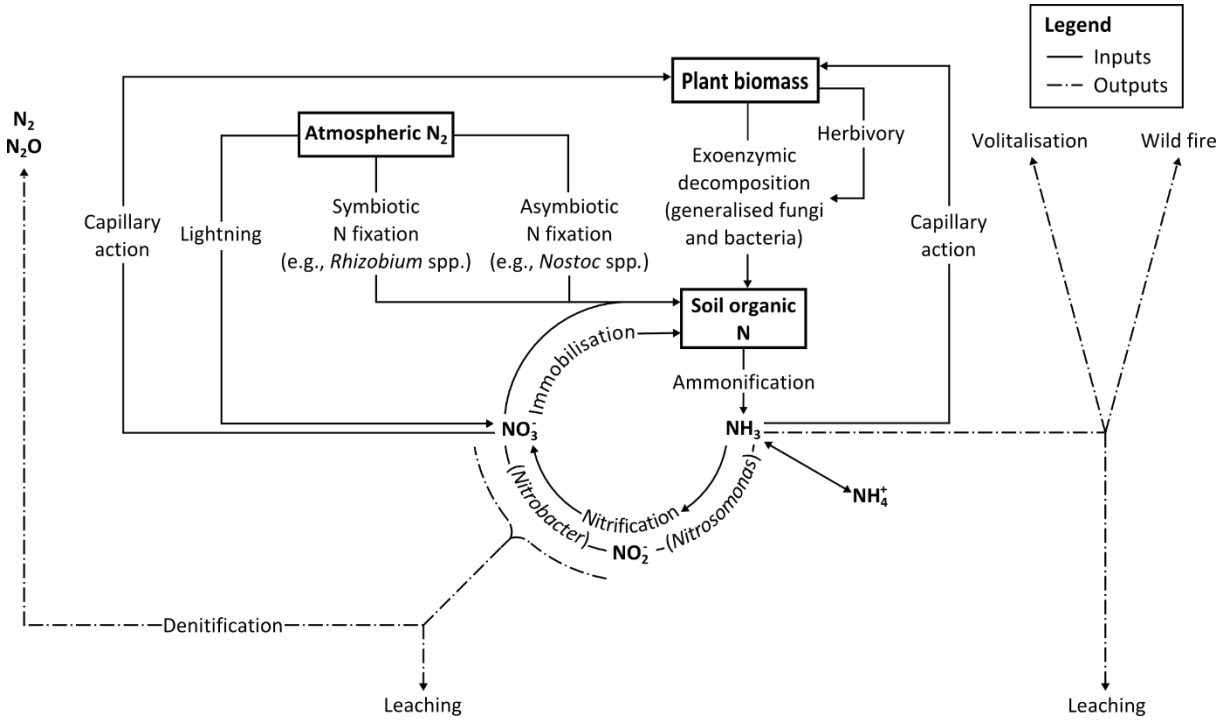

Figure 1.1 A schematic outlining the nitrogen (N) cycle in unmanaged terrestrial ecosystems (adapted from Stevenson, 1982).

Soil N exhibits high spatial heterogeneity in arid ecosystems, where concentrations are greater in vegetated soils as opposed to bare ground (Housman *et al.*, 2007). Spatially discrete areas of enhanced N availability are primarily derived from above- and below-ground inputs of organic matter to vegetated soils. In addition, N may also be redistributed from bare interspaces to vegetated soils via aeolian and hydrological transport processes, as well as through extensive lateral root systems (Ravi *et al.*, 2010a; Schlesinger and Pilmanis, 1998; Turnbull *et al.*, 2011). As >90 % of soil N is typically contained within the organic phase (Stevenson, 1982), the elevated N content of soils beneath vegetated surfaces represent important stores of mineralisable N (Vitousek *et al.*, 2002b). Accordingly, the availability of N in dryland systems is strongly influenced by processes associated with decomposition (Delgado-Baquerizo *et al.*, 2015). However, N products resulting from the decomposition of organic matter are susceptible to gaseous losses by volatilisation and nitrification processes (Evans and Ehleringer, 1993). Fresh inputs of reactive N to dryland systems are supplied by N fixation processes (Vitousek *et al.*, 2002b).

The atmosphere, of which 78 % is comprised of N_2, contains 99.96 % of all non-crustal N on Earth (Mengel, 1996). However, since N_2 exhibits a formal charge of zero, it is largely inert within the environment (Oldroyd and Dixon, 2014). In order to enter the biological system, N_2 must first be converted to reactive chemical forms via fixation (Vitousek *et al.*, 1997b). The principal processes which fix N_2 in unmanaged arid ecosystems are lightning and the *in situ* biological-fixation of atmospheric N_2, for which the quantity of available N provided by biological N fixation (BNF) is approximately an order of magnitude greater than that which is produced by lightning (Cleveland *et al.*, 1999).

BNF is catalysed by the nitrogenase enzyme system which mediates the adenosine triphosphate (ATP)-reduction of N_2 to the metabolically available form of NH_3 according to the stoichiometry:

$$8H^+ + 8e^- + N_2 + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i \quad \textbf{(1.1)}$$

where; e is electron, MgATP is magnesium-ATP, MgADP is magnesium-adenosine diphosphate and P_i is inorganic phosphate. The nitrogenase enzyme is limited to a diverse group of asymbiotic (i.e., free-living) and symbiotic prokaryotes termed diazotrophs (Kim and Rees, 1994). Whilst the relative contributions of these inputs may vary, rates of BNF by symbiotic plant-microbe associations are approximately an order of magnitude greater than inputs by free-living soil archaea and bacteria (Cleveland *et al.*, 1999). For arid ecosystems in polar and non-polar regions, rates of asymbiotic and symbiotic BNF are estimated to range from 0.42-13 kg N ha^{-1} a^{-1} and 0.67-97.5 kg N ha^{-1} a^{-1}, respectively (Cleveland *et al.*, 1999). However, the variation between the extrema of these estimates, which span an order of magnitude, suggest that the biological fixation of N is sensitive to external environmental parameters.

Inputs of N through asymbiotic pathways have the potential to exceed total inputs of N-containing compounds via atmospheric deposition, which are often low in drylands (e.g., 1-2 kg N ha^{-1} a^{-1} for drylands in southwestern US; Schwede and Lear, 2014), due to a remoteness from anthropogenic influences (Evans and Ehleringer, 1993). Thus, despite exhibiting lower rates of fixation in comparison to plant-microbe associations, asymbiotic BNF can represent an important source of biologically available N to nutrient-limited dryland soils (Bradley *et al.*, 2014). However, it is expected that rates of BNF will down-regulate as concentrations of soil N increase relative to the availability of phosphorus (P) as a result of the high demand of the latter for ATP (Menge and Hedin, 2009). Yet the factors influencing the extent and distribution of asymbiotic BNF in arid ecosystems are poorly resolved.

The availability of inorganic N, which is defined hereinafter as the sum of N as ammonium (NH_4^+-N) and nitrate (NO_3^--N), is not directly influenced by BNF as inputs of N through these asymbiotic and symbiotic pathways are assimilated into glutamate or absorbed into the vascular systems of plant symbionts, respectively (Knowles, 2009). Instead, inorganic N is derived from mineralisation processes which are influenced by the stoichiometry between soil organic carbon (SOC) and total N (Hassink, 1994; Vitousek *et al.*, 2002b). Thus, N is liberated when N is in excess and immobilised when N is limiting (Delgado-Baquerizo *et al.*, 2015). As the cycles of C and N are projected to become uncoupled with increases in aridity (Delgado-Baquerizo *et al.*, 2013) and inorganic N deposition (Thornton *et al.*, 2007), advancing knowledge of the controls on the distribution and speciation of soil N in arid ecosystems is of critical concern for global biogeochemical cycles.

1.1.3 Environmental change and soil nitrogen in arid ecosystems

Arid ecosystems are undergoing environmental changes in response to shifting climate and land-use dynamics (Maestre *et al.*, 2012b). As the N cycle is mediated almost exclusively by microbiological processes (Robertson and Groffman, 2015), climate-driven changes in water availability may perturb N input and transformation rates (Delgado-Baquerizo *et al.*, 2013). Further, as vegetation controls the quality and quantity of organic N inputs (Vinton and Burke, 1995), and modulates the degree of environmental stresses on the microbial community, changes in surface-cover type with alterations in land use have the potential to affect the spatial distribution and fluxes of soil N (Creamer *et al.*, 2016). As arid ecosystems, which support >2.5 billion people (Reynolds *et al.*, 2007b), are projected to expand to occupy ~50 % of Earth's land surface in response to climate warming by 2100 (Huang *et al.*, 2016), there is a need to improve understanding of how the soil N pool will respond to environmental changes.

Environmental change in the form of land degradation (i.e., a decline in the productive capacity of an ecosystem) is estimated to have occurred in 10-20 % of Earth's dryland area, for which the socioeconomic consequences have affected ~250 million people (Reynolds *et al.*, 2007b). Over the past

ca. 150 years, the prevailing form of land degradation within arid grasslands, which comprise >30 % of the continental surface and account for 30-35 % of terrestrial net primary production, has been associated with woody plant encroachment by shrub functional types (Knapp *et al.*, 2008; Ravi *et al.*, 2010a). The proliferation of, typically indigenous, C_3 woody plants into C_4-dominated grasslands is frequently accompanied by a reduction in the cover and number of herbaceous species, and changes in ecosystem functioning (D'Odorico *et al.*, 2012). This phenomenon, which is considered irreversible on human timescales, is widely attributed to changes in land use (Kröel-Dulay *et al.*, 2015) and has serious implications for pastoral enterprises, as forage for livestock is replaced by unpalatable shrubs (D'Odorico *et al.*, 2012). Further, such changes in plant community composition have been shown to significantly perturb the spatial distribution of soil resources, as well as the biogeochemical cycling and transport of nutrients in and between soils under shrub canopy and intershrub cover types (e.g., Brazier *et al.*, 2014; Eldridge *et al.*, 2011; Schlesinger *et al.*, 1996; Turnbull *et al.*, 2011; Turnbull *et al.*, 2010). Specifically, reductions in herbaceous cover increase the connectivity of aeolian and hydrologic erosion pathways within landscapes, which promotes the redistribution of nutrients adsorbed onto fine soil particles from intershrub areas to zones beneath shrub canopies (Figure 1.2; Michaelides *et al.*, 2012; Ravi *et al.*, 2010a). The resulting accumulations of nutrients in soils under shrub canopies is argued to benefit the persistence of shrub functional types at the expensive of herbaceous biomass (Schlesinger *et al.*, 1996). Consequently, soil C, N and P pools, which regulate plant and microbial stoichiometry, are strongly influenced by the expansion of woody plant species (Browning *et al.*, 2008). For more information concerning the impacts of woody plant encroachment on the soil N pool in arid ecosystems, see section 4.2.

In contrast to arid grasslands, environmental change in similarly water-limited high latitude and altitude ecosystems, which may be considered analogous extensions of drylands (see section 1.1.4), is largely driven by rising atmospheric temperatures (Stocker *et al.*, 2013). Warming-related declines in snow and ice cover since ca. 1850 have progressively exposed primary mineral substrates to soil-forming conditions (Breen and Lévesque, 2008). The subsequent development of these substrates into soils occurs over long timescales as soil-forming processes are inhibited by the low near-surface temperatures, short growing seasons and slow weathering rates common in high latitude regions (Ellis and Mellor, 1995). Consequently, the nutrient status of glacier forefield soils is initially low, where concentrations of SOC, total N and total P are in the range of 0.1-40 mg g^{-1}, 0.1-2 mg g^{-1} and 2-8 µg g^{-1}, respectively (Bradley *et al*., 2014). Whilst inputs of P to forefield soils are largely derived from the weathering of mineral substrates, C and N are obtained from autochthonous microbial processes (e.g., photosynthesis for C and BNF for N), wet and dry deposition, and the release of ancient organic matter from surfaces previously overridden by ice (Figure 1.3). These inputs of C and N may then be redistributed through the landscape by aeolian and hydrological processes (Bradley *et al.*, 2015). Due

to the low concentrations of N in recently exposed soil-forming substrates (Vitousek and Farrington, 1997), the colonisation of the soil is mostly restricted to asymbiotic diazotrophs (Brankatschk *et al.*, 2011). Inputs of N by asymbiotic BNF, as well as atmospheric deposition, to high altitude and latitude soil systems then facilitates the colonisation of the surface by plants, which are capable of increasing soil C and N pools through inputs of nutrient-rich litter and root exudates (Bradley *et al.*, 2014; Duc *et al.*, 2009). Further, successional changes in plant community composition have been shown to select the microbiota responsible for nutrient cycling in the rhizosphere (Mapelli *et al.*, 2018). For more information concerning the effects of declining ice cover on the soil N pool in water-limited ecosystems, see section 3.2.

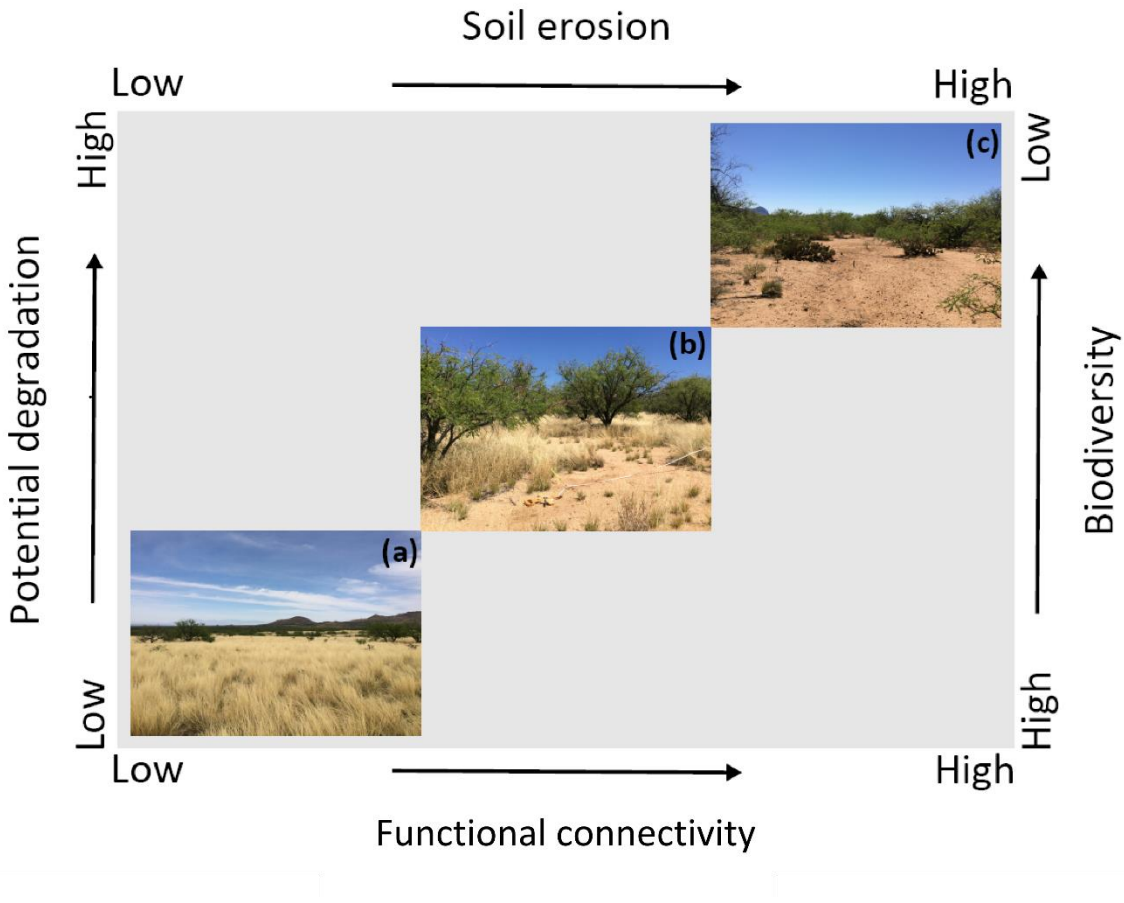

Figure 1.2 Conceptual diagram detailing changes in biodiversity, functional connectivity and soil erosion with the transition of a grassland (a), to a shrub-encroached grassland and finally (b), to a shrubland (c) in the Santa Rita Experimental Range, AZ, US (adapted from Ravi *et al.*, 2010a; photographs by T. Turpin-Jelfs).

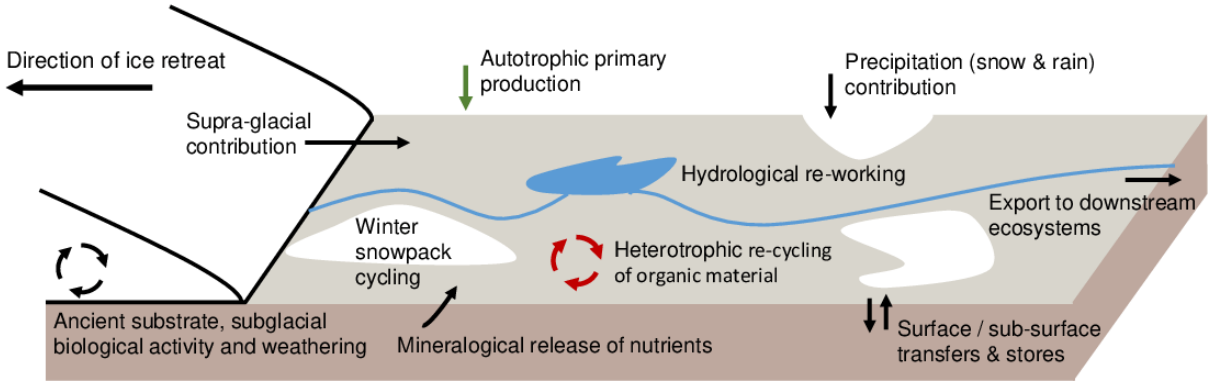

Figure 1.3 Conceptual diagram illustrating the major biotic and abiotic processes regulating inputs and transformations of nutrients in glacier forefield ecosystems (image from Bradley *et al.*, 2015).

Knowledge of how environmental changes associated with declining ice cover and the proliferation of woody plants affect the status and cycling of N in water-limited ecosystems is incomplete (Bradley *et al.*, 2014; Browning *et al.*, 2008). Further, a comprehensive understanding of how changes within the soil N pool vary between trajectories of succession and degradation is missing. Due to the importance of N to soil fertility and ecosystem productivity, a study is required to assess and compare the impacts of ecosystem transformations N input processes, such as BNF, the major source of new N to unmanaged drylands, as well as the distribution and speciation of N in and between emerging and degrading arid ecosystems (Figure 1.4). As the biogeochemical cycling of N is sensitive to temperature and water availability (Wang *et al.*, 2014), it is important that such a study should consider how the soil N pool responds to environmental changes across a spectrum of aridity.

1.1.4 Defining aridity

Drylands are water-limited ecosystems which are characterised by low levels of annual precipitation and an uneven distribution of water (Noy-Meir, 1973). Thus, it is in relation to aridity, as defined by a paucity of water, that drylands are frequently classified (Parsons and Abrahams, 2009a). However, a scientific consensus regarding an appropriate measure of aridity by which to determine a dryland has yet to be established, where it is common for researchers to classify these systems in accordance with their respective disciplines (Gamo *et al.*, 2013; Tucker *et al.*, 1991). Consequently, whilst the general locations of arid regions are widely accepted, there is disagreement concerning the methods used to delineate boundaries between areas that differ in degrees of aridity (Maliva and Missimer, 2012).

The simplest index of aridity classifies arid and semi-arid drylands as areas in which mean annual precipitation (MAP) is <250 mm and between 250 mm and <500 mm, respectively (Maliva and Missimer, 2012). However, as aridity is a function of both inputs of precipitation and outputs of water vapour to the atmosphere, it can be argued that the use of MAP as an index of aridity would fail to reflect important interactions between precipitation, temperature and evaporation (Stadler, 2005).

Thus, indices of aridity which consider inputs and outputs of moisture are more widely applied to the delineation of arid ecosystems (Maliva and Missimer, 2012).

Aridity is typically expressed as the ratio of MAP to potential evapotranspiration (PET; Maliva and Missimer, 2012), for which PET is estimated using air temperature and latitude data in accordance with methods outlined by Thornthwaite (1948). The most commonly cited aridity index founded on the methods developed by Thornthwaite (1948) was produced by the United Nations Environment Programme (UNEP, 1992) and defines drylands as areas which exhibit a MAP to PET ratio of <0.65. In addition, this classification system further delineates drylands with aridity indices of <0.05, 0.05 to <0.02, 0.02 to <0.5 and 0.5 to 0.65 as hyper arid, arid, semi-arid and dry sub-humid, respectively. The resulting sum of these areas is estimated to comprise 47.2 % (6.15 x 10^7 km^2) of the global land surface area. However, these classification criteria fail to consider drylands in high latitude regions which collectively cover an additional area of ~5 x 10^6 km^2 or ~3 % of Earth's terrestrial surface (Coble *et al.*, 1987; Cockell and Stokes, 2006).

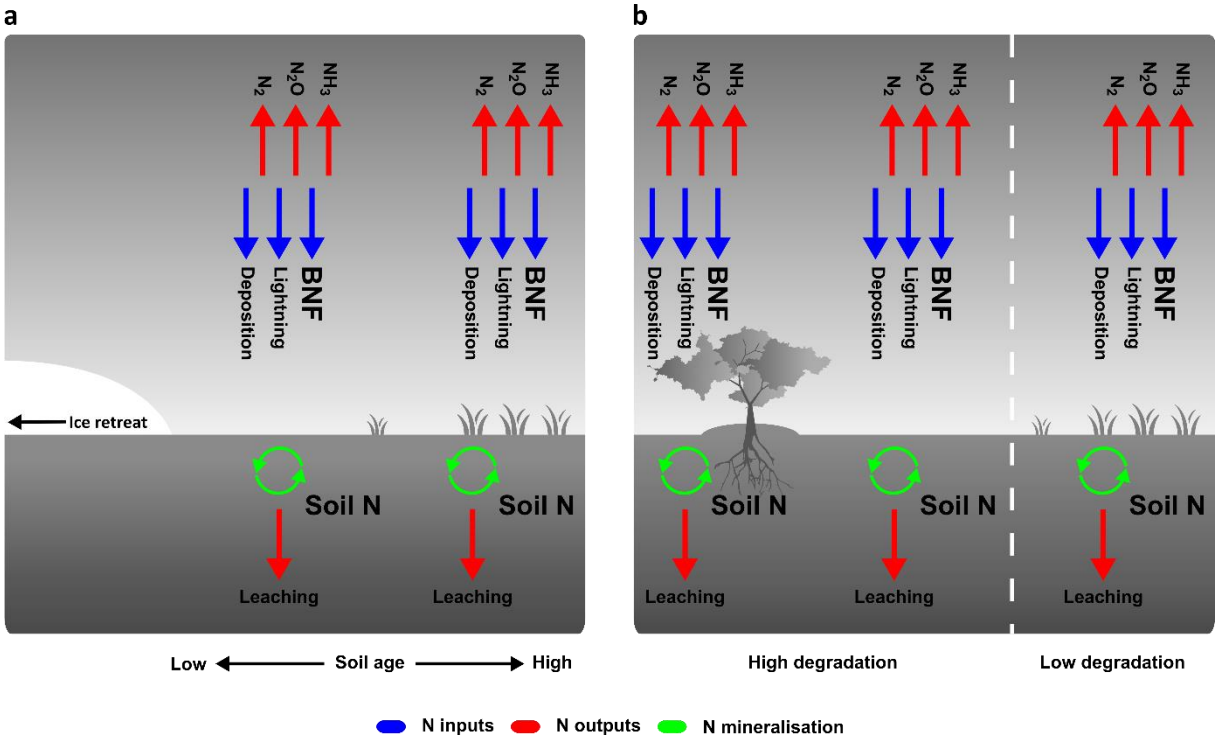

Figure 1.4 Conceptual model illustrating the inputs (wet and dry deposition, lightning and biological processes), transformations (mineralisation) and outputs (emissions of dinitrogen (N_2), nitrous oxide (N_2O) and ammonia (NH_3)) of nitrogen (N) in expanding glacier forefields (a) and grasslands undergoing shrub encroachment (b), for which this study will focus on the distribution of the soil N pool, as well as inputs of N by biological N fixation (BNF).

Drylands in high latitude regions are arbitrarily defined as glacier-free terrestrial areas where mean annual precipitation is <250 mm and mean temperature is <10 ˚C during the warmest month of the year (Cockell and Stokes, 2006). Such systems are not regarded as "true" drylands because conditions of aridity are not caused by deficiencies in the amount of water received as precipitation relative to that which is lost through evaporation. Instead, aridity occurs in response to the low surface temperatures and stable air masses prevalent throughout the year which limit evaporation processes and the formation of clouds, respectively. Further, precipitation received largely as snow increases the degree of aridity in polar regions as it may be unavailable to the biota in liquid form for long periods prior to the melt season (Logan, 1968). Consequently, like their non-polar counterparts, drylands in high latitude regions are examples of water-limited environments (Logan, 1968).

A world climate classification scheme developed by Köppen (1936), which is hereinafter referred to as the Köppen-Geiger climate classification scheme, is the most widely used system for classifying climates on Earth (Peel *et al.*, 2007). Recognising plant species ranges as indicators of climate, the system uses transitions between vegetation groups to delineate boundaries between the following five broad climate classes: tropical, arid, temperate, cold and polar (Kottek *et al.*, 2006). According to this climate classification scheme, drylands are defined as environments in which MAP is <10 times that of a dryness threshold, which can be calculated for an area using the following equation (1.2):

$$P_{threshold} = \begin{cases} 2\,(MAT) & \textit{if 70 \% of MAP occurs during winter} \\ 2\,(MAT) + 28 & \textit{if 70 \% of MAP occurs during summer} \\ 2\,(MAT) + 14 & \textit{or else} \end{cases} \quad \textbf{(1.2)}$$

where; $P_{threshold}$ is the dryness threshold (mm) and MAT is the mean annual near-surface (2 m) temperature (˚C). If arid ecosystems exhibit MAP that is ≥5 to <10 times a dryness threshold they are classed as arid steppe (BS); whereas arid desert ecosystems (BW) are classed as areas where MAP is <5 times a dryness threshold. Further, these ecosystems, which collectively cover 30.2 % of the total land area, can be subdivided into hot (h) or cold (k) drylands where MAT is ≥18 ˚C (i.e, BSh and BWh) or <18 ˚C (i.e., BSk and BWk), respectively. In contrast, polar climate zones, which cover 12.8 % of the total land area, are defined as areas where the temperature during the warmest month is <10 ˚C. Such climate zones may then be classed as polar tundra (ET) if the temperature of the warmest month is >0 ˚C to <10 ˚C or polar frost (EF) if the warmest month's temperature is ≤0 ˚C (Peel *et al.*, 2007).

If ice-free areas in the EF and ET climate classes are considered arid because they exhibit temperatures of <10 ˚C during their warmest months, it can be argued that the Köppen-Geiger climate classification scheme is useful for delineating zones of aridity in non-polar, as well as high latitude and altitude regions. Further, as different regions within the same climate class can be expected to contain similar vegetation, this climate classification scheme is useful for facilitating comparisons between the extents

of vegetation changes on the soil N pool between ecosystems. Thus, for the purposes of this research project, the following six zones of aridity were identified using a widely cited global map of climate produced by Peel *et al.* (2007): EF, ET, BSk, BSh, BWk and BWh (Figure 1.5). Peel *et al*. (2007) estimate the sum of the areas covered by these climate classes to encompass 43 % of the terrestrial surface. Ecosystems being exposed by glacial/ice recession or undergoing woody plant encroachment within any of these six zones of aridity are hereinafter referred to as emerging ice-free arid ecosystems and shrub-encroached drylands, respectively.

1.2 Aims and objectives

The main aim of this research project was to improve current understanding of how the soil N pool responds to environmental changes between trajectories of succession and degradation in arid ecosystems. The aim was addressed through the following specific objectives using a combination of fieldwork, laboratory experiments and desk-based research:

i. Using meta-analysis of published literature, to determine how the distribution and speciation of soil N responds to changes in surface cover associated with environmental changes between and among expanding high latitude and altitude glacier forefields (succession) and drylands undergoing shrub encroachment (degradation) globally;
ii. To identify how time on soil development since glacial retreat impacts the soil N status and inputs of N by asymbiotic BNF in a high latitude glacier forefield; and
iii. To determine how the soil N status and inputs of N by asymbiotic BNF change along a gradient of increasing shrub encroachment within an arid grassland.

1.3 Thesis structure

This thesis is structured as a collection of papers which pertain to each of the primary objectives that have been outlined. Chapter 1 is a general introduction which introduces the research topic and main objectives. Chapter 2 is a manuscript for a research article which addresses the first objective of this thesis by examining how the soil N pool responds to surface-cover changes in emerging and degrading arid ecosystems worldwide using meta-analysis of published data. Chapter 3 is a manuscript for a research article which addresses the second objective of this thesis by exploring how the distribution and speciation of N, as well as rates of asymbiotic BNF, change with time on soil development in a High Arctic glacier forefield. Chapter 4 is a manuscript for a research article which addresses the third objective of this thesis by investigating how the distribution and speciation of soil N, as well as rates of asymbiotic BNF, change along a gradient of increasing shrub cover in an arid grassland. Lastly, Chapter 5 is a general discussion which synthesises the main findings from chapters 2, 3 and 4 and identifies

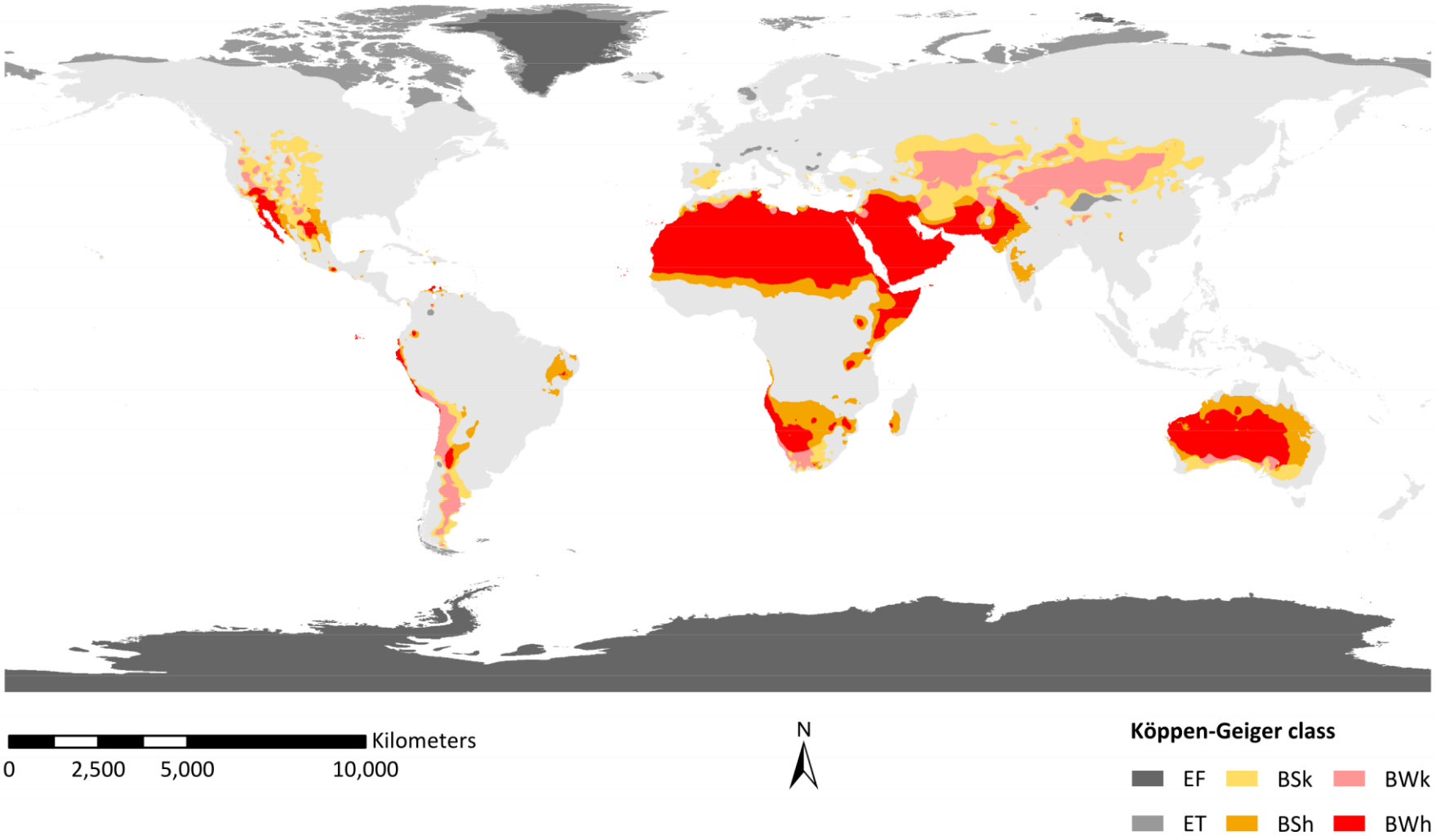

Figure 1.5 Köppen-Geiger climate type map for the following classes of aridity as determined by Peel *et al*. (2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot arid steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh).

areas of further research concerning the impacts of environmental change on the soil N pool in emerging ice-free and shrub-encroached arid ecosystems. The methodologies employed to conduct this research are described in detail within each chapter. Further, information concerning author contributions and manuscript publications are detailed at the beginning of each chapter.

2 A meta-analysis to compare the effects of environmental change on the soil nitrogen pool in emerging and degrading arid landscapes

This chapter has been prepared as a research article manuscript for future submission to a peer-reviewed journal. Co-authors include Thomas Turpin-Jelfs,[1] Katerina Michaelides[1,2] and Alexandre M. Anesio[1,3]. It must be noted that sections 2.2 and 2.3.1 have been abridged to prevent the unnecessary repetition of information presented elsewhere in this thesis.

Thomas Turpin-Jelfs is the lead author and was responsible for designing the study, conducting the meta-analysis, and writing the manuscript. Katerina Michaelides and Alexandre M. Anesio are co-authors responsible for advising on the methodology and editing the manuscript prior to submission.

The article uses meta-analysis of published data to examine the response of the soil nitrogen pool to surface-cover changes in emerging ice-free arid ecosystems and shrub-encroached drylands and addresses the first objective of this thesis (see section 1.2).

[1] School of Geographical Sciences, University of Bristol, Bristol, BS8 1SS, UK

[2] Earth Research Institute, University of California Santa Barbara, Santa Barbara, CA, US

[3] Department of Environmental Science, Aarhus University, 4000 Roskilde, Denmark

2.1 Abstract

Shifts in climate and land-use dynamics over the past ca. 150 years have resulted in environmental changes in water-limited ecosystems worldwide. Declines in snow and ice cover resulting from rising temperatures are progressively exposing new soil forming mineral substrates to biotic colonisation in high latitude and altitude regions, whereas land-use changes in continuous water-limited grasslands are causing herbaceous species to be replaced by shrub functional types and bare-soil interspaces. If viewed as trajectories of succession and degradation, emerging ice-free arid ecosystems and shrub-encroached drylands provide unique opportunities to explore how nitrogen, the most significant limiting nutrient of primary production in arid ecosystems, responds to surface-cover changes in soil. Using meta-analysis, we determined that the total nitrogen pool of soils increases between bare soil and woody cover types in both emerging ice-free and shrub-encroached arid ecosystems. Yet whilst surface-cover changes in the order bare soil, non-vascular, herbaceous and woody can be considered a linear trajectory of succession in emerging ice-free arid ecosystems, the same cannot be said for degrading water-limited grasslands. If increases in woody cover are weighted against concurrent increases in bare soil in shrub-encroached drylands, we found that although the ecosystem-level total nitrogen pool remains constant with grassland-to-shrubland transitions, it is concentrated into soils beneath shrub canopies. This redistribution of nitrogen will favour the persistence of woody plants and result in the continued loss of herbaceous cover in these areas. Further, the total nitrogen content of soil under woody cover is lower in shrub-encroached drylands than emerging ice-free ecosystems. This suggests the emerging ice-free ecosystems have the potential to become more productive than shrub-encroached drylands with continued primary succession and soil development. However, when studies identified during the meta-analysis were delineated by degrees of aridity, soil carbon-to-nitrogen ratios for bare soil and non-vascular cover types in emerging ice-free arid ecosystems were determined to vary, which suggest soil development in high latitude and altitude regions may be sensitive climate perturbations. As emerging ice-free and shrub-encroached arid areas are projected to expand, this study has implications for understanding how surface-cover changes will modify ecosystem productivity in arid regions.

2.2 Introduction

The context and background information for this chapter can be found in section 1.1.3.

We have conducted a meta-analysis to establish and compare how concentrations of total nitrogen (N), ammonium (NH_4^+-N) and nitrate (NO_3^--N), as well ratios of SOC to total N (C:N hereinafter), respond to changes in cover type in emerging ice-free arid ecosystems (i.e., ecosystems being exposed by glacial/ice recession) and shrub-encroached drylands (i.e., ecosystems undergoing woody plant encroachment) by varying degrees of aridity.

2.3 Materials and Methods

2.3.1 Defining aridity

Whilst a range of criteria may be used, drylands are typically defined in relation to aridity, which is a function of temperature and precipitation (Parsons and Abrahams, 2009b). In this study, the following six zones of aridity were identified using a global map of climate which had been produced in accordance with the Köppen-Geiger climate classification scheme by Peel *et al.* (2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot arid steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). For a discussion concerning the methods used to delineate boundaries of aridity, as well as a justification for applying the Köppen-Geiger climate classification scheme to this study, see section 1.1.4.

2.3.2 Search strategy and eligibility criteria

We compiled data from the literature concerning the effects of vegetation change on concentrations of total N, exchangeable NH_4^+-N and NO_3^--N, and C:N ratios in surface soils (depth of ≤15 cm) in emerging ice-free arid ecosystems (succession study class) and shrub-encroached drylands (degradation study class). Literature searches by topic without language restrictions were performed in Web of Science version 5.30 for the period 1900 to 21 December 2018. The following search terms and Boolean operators were combined to identify relevant studies pertaining to the succession study class: (glacier fore* OR ice-free OR polar desert) AND soil AND (biogeo* OR chemical properties OR *nitrogen* OR nitrogen input* OR nutrient*). Likewise, the following search terms and Boolean operators were used to find applicable studies for the degradation study class: (*arid OR desert OR dryland) AND (encroach* OR Invasion OR proliferation) AND soil AND (biogeo* OR chemical properties OR *nitrogen* OR nitrogen input* OR nutrient*). Literature searches returned 434 and 436 studies for the succession and degradation study classes, respectively. All retrieved references were screened for relevance by title, abstract and Köppen-Geiger climate class (climate class hereinafter). To be included in our analysis, studies had to contain replication, state the cover type (all cover types did not have to be present within a single study) of sampling locations and report results on a dry weight basis (data from review articles and model-based analyses were excluded from this study). Accepted results were ordered according to study class, cover type and climate class. Cover types were categorised as bare soil, non-vascular (e.g., biological soil crusts, lichens and mosses), herbaceous (e.g., forbs and graminoids) and woody (e.g., shrubs and trees). Where necessary, data values were extracted from plots electronically using the Figure_Calibration plug-in for ImageJ version 1.46 developed by Hessman (2009). See Table 2.1 for a summary concerning the number of accepted data sources for total N, NH_4^+-N and NO_3^--N, and C:N ratios in surface soils by climate class for both the succession and degradation study classes. Please note, to maximise the data available for subsequent meta-analysis,

results for the variables of interest were accepted according to their operational definitions and not the methods used in their determination.

2.3.3 Calculations

SOC and total N data were used to calculate atomic C:N ratios. In studies where SOC was not directly reported, SOC (μg g^{-1}) was estimated from percentage organic matter content (%OM) data when available using the equation (2.1):

$$SOC = \%OM \times 0.5 \times 10^4 \quad \textbf{(2.1)}$$

where carbon was assumed to comprise half of all organic matter (Pribyl, 2010).

2.3.4 Statistical analysis

Statistical analysis of the data was performed using R version 3.5.0. Tests of significance were carried out to determine if soil N concentrations or C:N ratios differed between (i.e., between cover types within the same climate or study class) or within (i.e., between climate or study classes within the same cover type) groups. The Mann-Whitney *U* (MW) and Kruskal-Wallis *H* (KW) tests were used to compare data between two and more than two groups, respectively. When significance was indicated by the KW test, Dunn's test (DT) of multiple comparisons was applied (Crawley, 2005). As the *H* test statistic poorly approximates a chi-squared distribution when sample sizes are too small, groups with $n < 5$ were excluded from KW and DT analyses (McDonald, 2008). In addition, the MW test was not used to analyse groups with $n < 5$ to minimise the risk of making false inferences from resulting *p* values (de Winter, 2013). For this study, the alpha level was set to 0.05. All errors reported in the text are one median absolute deviation about the median.

2.4 Results

2.4.1 Soil nitrogen with succession

From the literature search, we identified 70 relevant studies which reported data relating to soil N concentrations and/or C:N ratios by cover type for emerging ice-free arid ecosystems (see Figure 2.1 for study locations and Table 2.2 for data sources). Of the data collected, 38 %, 54 %, 7 % and 1 % originated from study locations within polar frost, polar tundra, cold arid steppe and cold arid desert climate classes, respectively. No data were identified for the hot arid steppe and hot arid desert climate classes.

Overall, the median total N content of soils from emerging ice-free arid ecosystems increases by cover type in the order bare soil < non-vascular < herbaceous < woody (Figure 2.2a). Specifically, concentrations of total N increase from 78 ±52 μg g^{-1} and 80 ±30 μg g^{-1} in bare soils to 1,555 ±1,370 μg g^{-1} and 470 ±210 μg g^{-1} in herbaceous soils for the polar frost (DT, $p < 0.001$) and cold

Table 2.1 Number of data sources for total nitrogen (N), ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) concentrations, as well as soil organic carbon-to-total N (C:N) ratios in soils from emerging ice-free arid ecosystems (succession study class) and shrub-encroached drylands (degradation study class) included within this meta-analysis by the following Köppen-Geiger climate classes as defined by Peel *et al.* (2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot arid steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). See tables 2.2 and 2.3 for data sources relating to the succession and degradation study classes, respectively.

	Study class											
	Succession						Degradation					
Data	**EF**	**ET**	**BSK**	**BWk**	**BSh**	**BWh**	**EF**	**ET**	**BSK**	**BWk**	**BSh**	**BWh**
Total N	29	47	6	1	0	0	0	0	22	2	15	10
C:N ratio	32	30	6	0	0	0	0	0	23	2	11	8
NH_4^+-N	18	22	2	0	0	0	0	0	6	0	4	0
NO_3^--N	19	22	2	1	0	0	0	0	8	0	3	0

arid steppe (MW, $p < 0.001$) climate classes, respectively, 140 ±130 μg g^{-1} to 1,430 ±1,235 μg g^{-1} between bare and woody polar tundra soils (DT, $p < 0.001$), and 5,400 ±2,500 μg g^{-1} in herbaceous to 9,500 ±600 μg g^{-1} in woody cold arid desert soils (MW, $p < 0.05$). Median total N concentrations for soils belonging to bare (KW, $p > 0.05$), non-vascular (MW, $p > 0.05$) and herbaceous cover types (KW, $p > 0.05$) do not differ among the climate classes analysed. However, the total N content of herbaceous cold arid desert soils, which were not included in the KW analysis ($n < 5$), appear greater than the contents of other soils within this cover type. Further, concentrations of total N in woody cold arid desert soils appear greater than those in woody soils within the polar tundra climate class.

Whilst C:N ratios remain unchanged for soils under different cover types in the polar tundra (KW, $p > 0.05$) and cold arid desert ($n < 5$) climate classes, C:N ratios increase from 9 ±3 to 22 ±12 and 8 ±2 to 14 ±3 between bare (DT, $p < 0.001$) and herbaceous (MW, $p < 0.001$) soils in the polar frost and cold arid steppe climate classes, respectively (Figure 2.2b). By cover type, polar tundra soil C:N ratios are greater in comparison with polar frost (DT, $p < 0.001$) and cold arid steppe (DT, $p < 0.001$) soils under the bare cover type, and polar frost soils under the non-vascular cover type (MW, $p < 0.001$). Conversely, soils originating from the polar frost, polar tundra and cold arid steppe climate classes exhibit similar C:N ratios (KW, $p < 0.05$). Finally, the woody cold arid desert soil C:N ratio does not appear to differ from the woody polar tundra and herbaceous cold arid desert soil C:N ratios.

The effect of cover type on the soil NH_4^+-N pool varies by climate class (Figure 2.2c). For cold arid steppe soils, concentrations of NH_4^+-N remain constant at ~1 μg g^{-1} between bare and herbaceous soils (MW, $p > 0.05$). Similarly, the NH_4^+-N contents of bare and herbaceous polar frost soils do not differ significantly from one another (DT, $p > 0.05$); however, the NH_4^+-N content of non-vascular soils is lower than both the bare (DT, $p < 0.05$) and woody (DT, $p < 0.001$) soils in this climate class. In contrast, concentrations of NH_4^+-N increase steadily from 1 ±0.5 μg g^{-1} to 5 ±2 μg g^{-1} between bare and herbaceous soils in the polar tundra climate class (DT, $p < 0.001$). By cover type, only the NH_4^+-N content of non-vascular soils appears to differ by cover type, for which concentrations were greater in polar tundra ($n < 5$) relative to polar frost soils.

Like the NH_4^+-N pool, the effect of cover type on concentrations of soil NO_3^--N differs among climate zones in arid ecosystems (Figure 2.2d). The NO_3^--N content of cold arid steppe soils remain constant at ~0.2 μg g^{-1} between bare and herbaceous cover types (MW, $p > 0.05$). Concentrations of NO_3^--N also remain constant at ~0.1 μg g^{-1} between bare, herbaceous and woody cover types within the polar tundra climate class (KW, $p > 0.05$); however, the NO_3^--N content of non-vascular soils within this climate class, which were not included in the KW analysis ($n < 5$), appear greater relative to the other soils within this climate class. In contrast, non-vascular soils exhibit a lower NO_3^--N content than bare (DT, $p < 0.05$) and woody (DT, $p < 0.001$) soils in the polar frost climate class. Finally, concentrations of

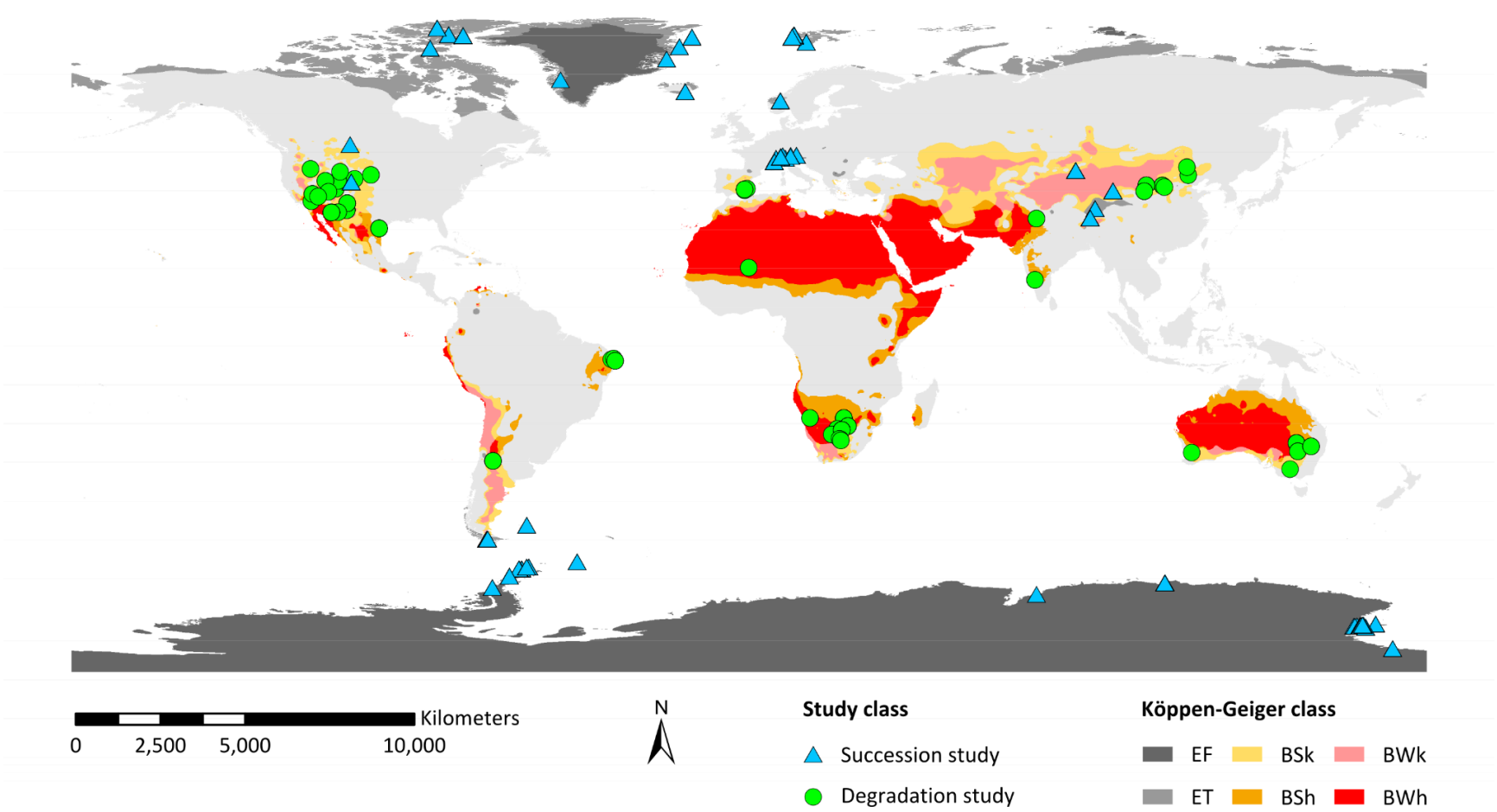

Figure 2.1 Locations of studies identified by meta-analysis detailing changes in the soil nitrogen pool with changes in vegetation in emerging ice-free (succession) and shrub-encroached (degradation) terrestrial arid ecosystems, respectively. Studies were limited to areas pertaining to the following Köppen-Geiger climate classes as determined by Peel *et al.* (2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot arid steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). See tables 2.2 and 2.3 for succession and degradation data sources, respectively.

Table 2.2 Data sources for total nitrogen (TN), ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) concentrations, as well as soil organic carbon-to-total nitrogen (C:N) ratios for soils from emerging ice-free arid ecosystems by Köppen-Geiger climate class as defined by Peel *et al.* (2007).

Location	°N	°E	TN	NH_4^+-N	NO_3^--N	C:N	Citation
Cold arid steppe (BSk)							
Canada	49.98	-105.92	●	●	●	●	Anaka *et al.* (2008)
China	30.48	90.63	●			●	Liu *et al.* (2016)
	43.10	86.80	●			●	Wu *et al.* (2018)
			●			●	Zeng *et al.* (2015)
			●			●	Zhang *et al.* (2016a)
	43.10	86.82	●	●	●	●	Zeng *et al.* (2016)
Cold arid desert (BWk)							
China	37.68	96.70	●			●	Tai *et al.* (2013)
Polar frost (EF)							
Antarctica	-83.75	170.98	●	●	●	●	Sokol *et al.* (2013)
	-78.08	163.82	●			●	Lee *et al.* (2011)
	-77.87	160.98	●	●	●	●	Barrett *et al.* (2007)
	-77.83	160.67	●	●	●	●	Barrett *et al.* (2007)
	-77.82	160.98				●	Bate *et al.* (2008)
	-77.73	162.32	●	●	●	●	Geyer *et al.* (2017)
	-77.72	161.52	●	●	●	●	Barrett *et al.* (2007)
	-77.72	162.32	●	●	●	●	Barrett *et al.* (2007)

Table 2.2 continued.

	-77.72	162.50				●	Bate *et al.* (2008)
			●			●	Hopkins *et al.* (2006)
	-77.63	162.87				●	Bate *et al.* (2008)
	-77.63	162.88	●	●	●	●	Barrett *et al.* (2007)
	-77.63	163.10	●			●	Barrett *et al.* (2004)
			●			●	Gooseff *et al.* (2003)
	-77.62	163.23	●	●	●	●	Aanderud *et al.* (2018)
	-77.62	163.25	●		●	●	Ball and Virginia (2014b)
			●	●	●	●	Barrett *et al.* (2007)
			●			●	Geyer *et al.* (2013)
			●	●	●	●	Sokol *et al.* (2013)
	-77.60	163.27	●	●	●	●	Ball and Virginia (2014a)
						●	Bate *et al.* (2008)
	-77.50	163.00		●	●		Zeglin *et al.* (2009)
	-77.25	166.48		●	●		Ball *et al.* (2015)
	-69.37	76.38	●			●	Shi *et al.* (2018)
	-67.57	-68.13	●	●	●	●	Yergeau *et al.* (2007)
	-66.37	110.50	●			●	Hovenden and Seppelt (1995)
	-64.77	-64.07	●			●	Beyer *et al.* (2000)
	-62.68	-60.85	●			●	Strauss *et al.* (2009)

Table 2.2 continued.

			●			●	González-Guzmán *et al.* (2017)
	-60.72	-45.63	●	●	●	●	Yergeau *et al.* (2007)
South Shetland Islands	-62.63	-60.35	●				Ganzert *et al.* (2011)
	-62.62	-61.10	●			●	Navas *et al.* (2008)
	-62.15	-58.45	●	●	●	●	Lachacz *et al.* (2018)
	-62.13	-59.03	●	●	●	●	Wang *et al.* (2015)
Falkland islands	-51.00	-59.00	●	●	●	●	Yergeau *et al.* (2007)
Polar tundra (ET)							
Austria	46.83	11.05	●	●	●	●	Tscherko *et al.* (2003)
			●	●	●	●	Tscherko *et al.* (2004)
			●	●	●	●	Tscherko *et al.* (2005)
	47.03	11.08	●	●	●	●	Hofmann *et al.* (2016)
	47.12	12.63	●	●	●	●	Tscherko *et al.* (2003)
Canada	75.55	-84.67	●	●	●	●	Anaka *et al.* (2008)
			●			●	Bliss *et al.* (1994)
	78.85	-75.90	●			●	Muller *et al.* (2017)
	78.88	-75.92	●			●	Bliss *et al.* (1994)
	79.17	-79.75	●			●	Breen and Lévesque (2008)
	80.88	-82.82	●			●	Osono *et al.* (2016)
Chile	-54.77	-69.67	●				Arróniz-Crespo *et al.* (2014)

Table 2.2 continued.

			●	●	●		Fernandez-Martinez *et al.* (2016)
	-54.68	-69.38	●			●	Pérez *et al.* (2017)
China	33.00	91.97	●			●	Liu *et al.* (2012)
France	45.43	6.77	●			●	Schwob *et al.* (2017)
Greenland	67.15	-50.05	●			●	Nash *et al.* (2018)
	72.67	-21.87	●			●	Ganzert *et al.* (2014)
	75.92	-18.45	●			●	Ganzert *et al.* (2014)
Iceland	64.00	-16.92	●			●	Vilmundardóttir *et al.* (2014)
			●			●	Vilmundardóttir *et al.* (2015)
Norway	61.58	8.35	●			●	Darmody *et al.* (2005)
Svalbard	77.08	15.25	●			●	Kabala and Zapart (2012)
	78.48	-15.12	●			●	Górniak *et al.* (2017)
	78.50	11.50	●				Fujiyoshi *et al.* (2011)
			●				Nakatsubo *et al.* (2010)
			●			●	Yoshitake *et al.* (2007)
	78.65	12.07	●			●	Wietrzyk *et al.* (2018)
	78.90	12.10	●			●	Kim *et al.* (2017)
	78.92	11.87	●	●	●	●	Hayashi *et al.* (2016)
			●	●	●	●	McCann *et al.* (2016)
	79.00	12.00	●			●	Yoshitake *et al.* (2018)

Table 2.2 continued.

Switzerland	46.32	7.22	●	●	●		Duc *et al.* (2011)
			●	●	●		Lazzaro *et al.* (2009)
			●	●	●		Lazzaro *et al.* (2012)
	46.32	9.70	●	●	●		Lazzaro *et al.* (2009)
	46.63	8.45	●	●	●	●	Brankatschk *et al.* (2011)
			●	●	●		Duc *et al.* (2009)
			●	●	●		Duc *et al.* (2011)
			●				Göransson *et al.* (2011)
			●	●	●		Lazzaro *et al.* (2009)
			●	●	●		Lazzaro *et al.* (2012)
				●	●		Miniaci *et al.* (2007)
				●	●		Noll and Wellinger (2008)
			●			●	Smittenberg *et al.* (2012)
			●				Zumsteg *et al.* (2013)
	46.82	8.42	●	●	●		Lazzaro *et al.* (2009)
	46.98	8.98	●	●	●		Lazzaro *et al.* (2009)
USA	40.05	-105.60	●				Liptzin and Seastedt (2009)

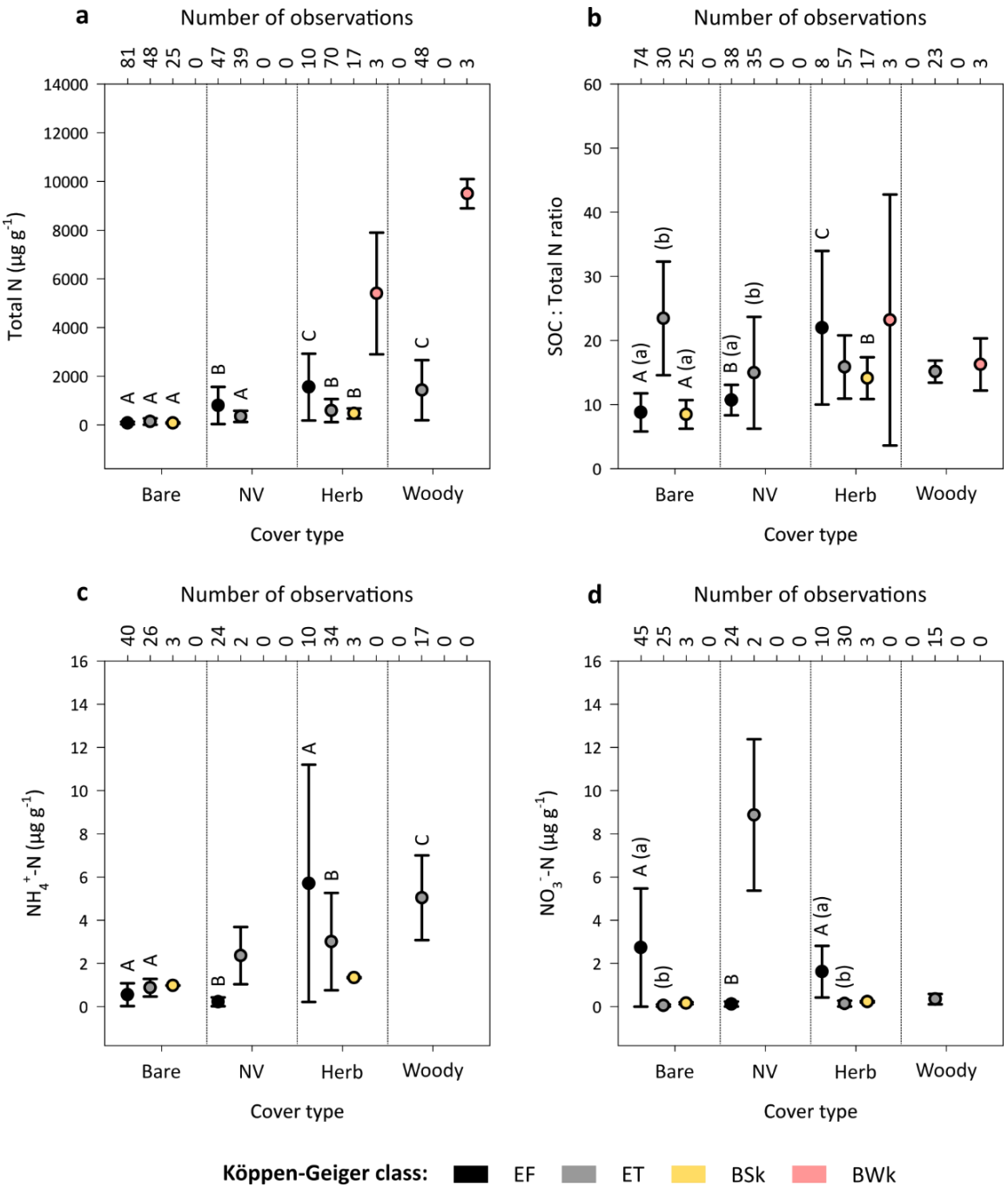

Figure 2.2 Median total N contents (a), SOC:total N ratios (b), NH_4^+-N contents (c) and NO_3^--N contents (d) identified by meta-analysis for surface soils (top ≤15 cm) under bare soil (bare), non-vascular (NV), herbaceous (herb) and woody cover types in emerging ice-free arid ecosystems belonging to the following Köppen-Geiger climate classes (Peel *et al.*, 2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot desert steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). No studies were identified for classes, BSh and BWk. See Table 2.2 for data sources and Appendix A for SOC data. Error bars represent one median absolute deviation about the median. Upper- and lower-case letters indicate significant differences at $p < 0.05$ within classes by cover type and between classes within cover types, respectively (Mann Whitney *U* test for two groups; Dunn's test for more than two groups; groups with n < 5 were excluded from tests of significance).

NO_3^--N were only determined to vary by climate class within the non-vascular cover type, where the polar tundra soil NO_3^--N content ($n < 5$) appears to be greater than that of the polar frost climate class.

2.4.2 Soil nitrogen with degradation

Of the studies returned during the literature search, 48 reported data relating to soil N concentrations and/or C:N ratios by cover type for shrub-encroached drylands (see Figure 2.1 for study locations and Table 2.3 for data sources). Data originating from locations within the cold arid steppe, hot arid steppe, cold arid steppe and cold arid desert climate classes comprised 48 %, 32 %, 3 % and 17 % of the studies identified, respectively. We found no data for the polar frost and polar tundra climate classes.

The effect of cover on soil total N in shrub-encroached drylands varies by climate class (Figure 2.3a). Within the hot arid steppe climate class, total N increases from 470 ±22 μg g^{-1} in bare soil to 845 ±341 μg g^{-1} in woody soil (DT, $p < 0.01$), but total N in herbaceous soils (700 ±177 μg g^{-1}) is not statistically separable from either bare (DT, $p > 0.05$) or woody soils (DT, $p > 0.05$). Similarly, total N increases from 358 ±156 μg g^{-1} in bare soil to 754 ±307 μg g^{-1} in woody soil within the cold arid steppe climate class (DT, $p < 0.001$); however, total N in herbaceous soil is greater than that in bare soil (DT, $p < 0.01$). In the cold and hot arid desert climate classes, total N ranges from 150 μg g^{-1} and 209 ±59 μg g^{-1} to 354 ±68 μg g^{-1} and 544 ±326 μg g^{-1} among cover types, respectively, in the sequence bare < herb < non-vascular < woody. Total N does not differ among climate classes for any cover type.

Overall, C:N ratios do not differ by cover type across the BSk (KW, $p > 0.05$) and BSh (MW, $p > 0.05$) climate classes (Figure 2.3b; BWk and BWh climate classes have groups with $n < 5$). Neither do C:N ratios vary among climate classes for the non-vascular (groups with $n < 5$), herbaceous (MW, $p > 0.05$) and woody (KW, $p > 0.05$) cover types, where the median C:N ratios for these cover types are 15, 13 and 12, respectively. However, the C:N ratio for bare cold arid desert soil is an order of magnitude lower than the median C:N ratios for other bare soils, as well as soils under different cover types within the same climate class ($n < 5$).

Despite only identifying data for the cold arid and arid desert climate classes, we determined that soil NH_4^+-N does not vary with changes in cover type (Figure 2.3c). Specifically, concentrations of NH_4^+-N range from 1 μg g^{-1} in bare soil to 3 ±1 μg g^{-1} in woody soil within the cold arid steppe climate class (MW, $p > 0.05$), whereas concentrations range from 20 ±19 μg g^{-1} in woody soil to 73 ±29 μg g^{-1} in non-vascular soil in the hot arid steppe climate class (MW, $p > 0.05$). However, whilst concentrations of NH_4^+-N do not differ among woody soils (MW, $p > 0.05$), the median NH_4^+-N content of the herbaceous hot arid steppe soil is an order of magnitude greater than that of the cold arid steppe soil ($n < 5$). Like the NH_4^+-N pool, concentrations of NO_3^--N do not differ among cover types for the cold arid steppe and hot arid steppe soils (Figure 2.3d). In cold arid steppe soils, concentrations of NO_3^--N range from

Table 2.3 Data sources for total nitrogen (TN), ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) concentrations, as well as soil organic carbon-to-total nitrogen (C:N) ratios for soils from ecosystems undergoing woody encroachment by Köppen-Geiger climate class as defined by Peel *et al.* (2007).

Location	°N	°E	TN	NH_4^+-N	NO_3^--N	C:N	Citation
Hot arid steppe (BSh)							
Australia	-31.50	145.82	●				Eldridge *et al.* (2015)
	-30.17	149.33	●				Good *et al.* (2012)
	-29.27	145.43	●			●	Daryanto *et al.* (2012)
			●			●	Daryanto *et al.* (2013)
Botswana	-22.65	25.23		●	●		Dougill and Thomas (2004)
				●	●		Dougill *et al.* (1999)
India	14.00	76.00	●			●	Chandregowda *et al.* (2018)
	30.28	76.38	●			●	Kaur *et al.* (2012)
South Africa	-28.18	24.07	●			●	Ward *et al.* (2018)
	-26.50	24.00	●				Hudak *et al.* (2003)
	-25.82	24.67		●			Thomas and Dougill (2006)
	-24.80	26.30	●				Hudak *et al.* (2003)
USA	27.67	-98.20	●			●	Boutton and Liao (2010)
			●			●	Hibbard *et al.* (2001)
			●			●	Liao and Boutton (2008)
			●			●	Zhou *et al.* (2018)
	31.90	-110.88				●	McClaran *et al.* (2008)

Table 2.3 continued.

			●			●	Wheeler *et al.* (2007)
	34.90	-116.20	●	●	●		Ewing *et al.* (2007)
Cold arid steppe (BSk)							
Argentina	-34.03	-67.97	●			●	Asner *et al.* (2003)
Australia	-36.20	143.75				●	Morgan *et al.* (2016)
	-31.80	117.60				●	Morgan *et al.* (2016)
China	38.65	110.38	●	●	●	●	Wei *et al.* (2013)
	39.03	109.85	●			●	Cheng *et al.* (2004)
	41.80	116.68	●			●	Li *et al.* (2017)
	43.92	116.33	●			●	Zhou *et al.* (2017)
South Africa	-28.60	24.45	●			●	Ward *et al.* (2018)
	-28.32	24.30	●			●	Ward *et al.* (2018)
Spain	37.82	-1.15	●			●	Rodríguez-Caballero *et al.* (2017)
	38.02	-1.15	●			●	Rodríguez-Caballero *et al.* (2017)
			●			●	Rodríguez-Caballero *et al.* (2017)
	38.07	-1.15	●			●	Rodríguez-Caballero *et al.* (2017)
	38.20	-0.50	●			●	Rodríguez-Caballero *et al.* (2017)
USA	31.95	-109.08		●	●		Wagner (1997)
	32.50	-106.78	●			●	Throop *et al.* (2013)
	32.55	-106.75	●			●	Alvarez *et al.* (2012)

Table 2.3 continued.

	34.32	-106.70	●		●	●	Turnbull *et al.* (2010)
	34.33	-106.72	●			●	Sankey *et al.* (2012)
	37.40	-111.68	●			●	Neff *et al.* (2009)
	38.17	-109.75	●				Evans and Belnap (1999)
	38.20	-111.17	●		●	●	Covert *et al.* (2015)
	40.28	-112.47	●	●	●	●	Hooker *et al.* (2008)
	40.28	-109.08		●	●		Hooker and Stark (2008)
	40.83	-104.72		●	●		Augustine *et al.* (2014)
	41.85	-100.37	●	●	●	●	Msanne *et al.* (2017)
	42.70	-108.60					Norton *et al.* (2008)
	43.45	-116.43	●			●	Eldridge and Whitford (2009)
Hot arid desert (BWh)							
Brazil	-7.43	-35.53	●			●	de Souza *et al.* (2018)
	-7.03	-36.50	●			●	de Souza *et al.* (2018)
	-6.85	-35.92	●			●	de Souza *et al.* (2018)
			●			●	de Souza *et al.* (2018)
Burkina Faso	17.28	-0.03	●				Traoré *et al.* (2015)
Namibia	-22.63	16.25	●			●	Wiegand *et al.* (2005)
South Africa	-27.00	22.00	●			●	Sandhage-Hofmann *et al.* (2015)
	-25.55	23.45	●			●	Hagos and Smit (2005)

Table 2.3 continued.

USA	36.10	-114.40	●		Suazo *et al.* (2012)
	36.82	-115.92	●	●	Schaeffer *et al.* (2003)
Cold arid desert (BWk)					
China	37.55	105.03	●	●	Song *et al.* (2017)
	39.13	105.58	●	●	Pei *et al.* (2006)

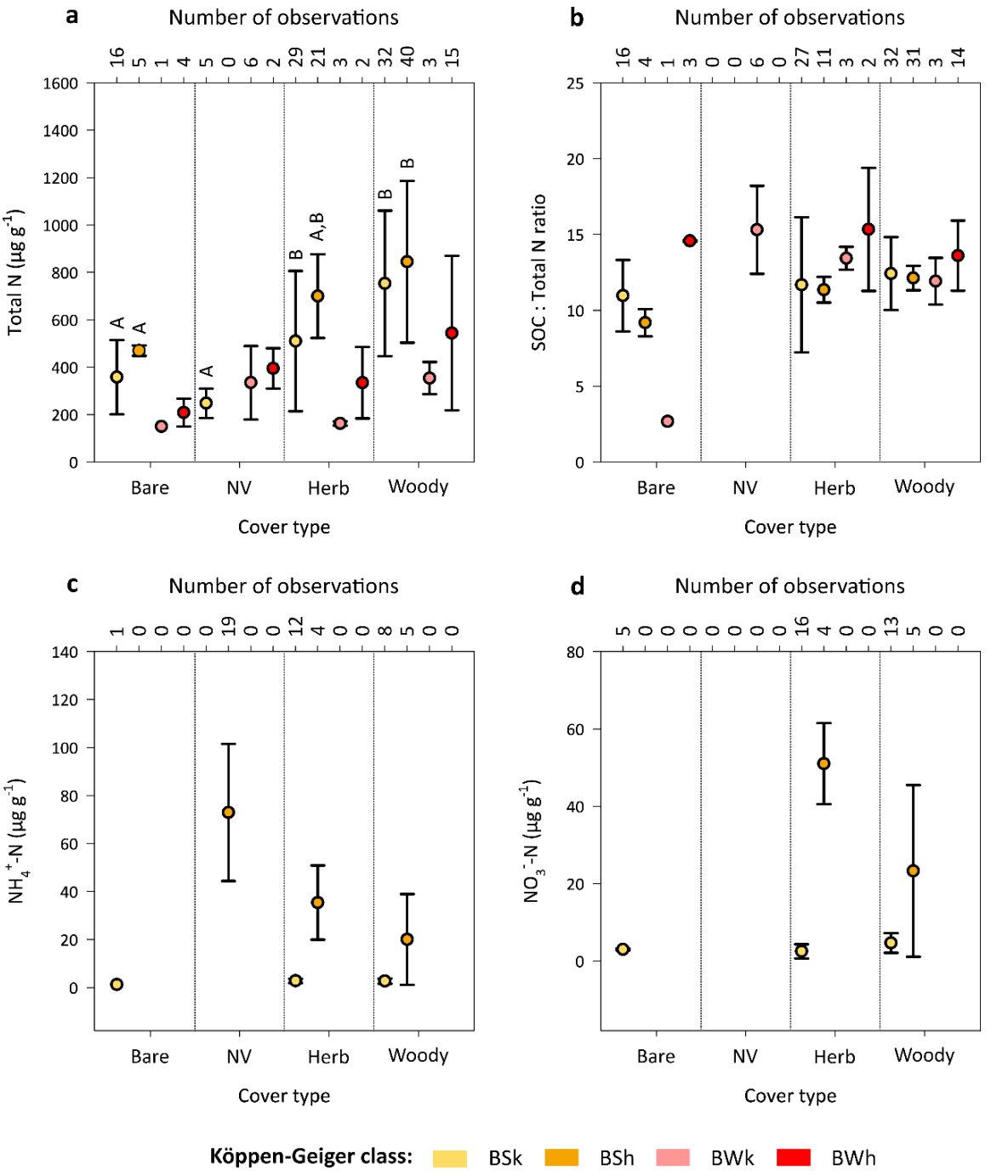

Figure 2.3 Median total N contents (a), SOC:total N ratios (b), NH_4^+-N contents (c) and NO_3^--N contents (d) identified by meta-analysis for surface soils (top ≤15 cm) under bare soil (bare), non-vascular (NV), herbaceous (herb) and woody cover types in shrub-encroached arid ecosystems belonging to the following Köppen-Geiger climate classes (Peel *et al.*, 2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot desert steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). No studies were identified for classes, EF and ET. See Table 2.3 for data sources and Appendix A for SOC data. Error bars represent one median absolute deviation about the median. Upper- and lower-case letters indicate significant differences at $p < 0.05$ within classes by cover type and between classes within cover types, respectively (Mann Whitney *U* test for two groups; Dunn's test for more than two groups; groups with n < 5 were excluded from tests of significance).

3 μg g^{-1} in bare and herbaceous soils to 5 ±3 μg g^{-1} in woody soils, whereas concentrations of NO_3^--N in hot arid steppe soils range from 23 ±22 μg g^{-1} to 51 ±10 μg g^{-1} between woody and herbaceous cover types, respectively. By cover type, concentrations of NO_3^--N in woody soils do not differ among climate classes (MW, $p > 0.05$); however, the NO_3^--N content of hot arid steppe soil is an order of magnitude greater than cold arid steppe soils under the herbaceous cover type ($n < 5$).

2.4.3 Soil nitrogen between trajectories of succession and degradation

Median global (median of all climate classes) concentrations of total N in both emerging ice-free and shrub-encroached arid ecosystems are greater in woody soils than bare soils (Figure 2.4a). For emerging ice-free arid ecosystems, total N increases from 90 ±66 μg g^{-1} in bare soil to 395 ±341 μg g^{-1} in non-vascular soil (DT, $p < 0.001$). Whilst total N does not differ between non-vascular and herbaceous soils in these emerging ice-free arid ecosystems (DT, $p > 0.05$), total N in woody soil is approximately five and three times greater than the concentrations observed in these non-vascular (DT, $p < 0.01$) and herbaceous soils (DT, $p < 0.05$), respectively. In shrub-encroached drylands, total N increases from 400 ±176 μg g^{-1} in bare soil to 584 ±302 μg g^{-1} in herbaceous soil (DT, $p < 0.01$). Total N in woody soils from these ecosystems subsequently increases above the levels present in herbaceous soil to 666 ±296 μg g^{-1} (DT, $p < 0.05$).

We observed total N in bare soils from shrub-encroached drylands exceeds total N in bare soil from emerging ice-free arid ecosystems (MW, $p < 0.001$; Figure 2.4a). Conversely, total N in woody soils from emerging ice-free arid ecosystems is greater than total N in woody soils from shrub-encroached drylands (MW, $p < 0.01$). Total N under non-vascular and herbaceous cover do not differ between emerging ice-free arid ecosystems and shrub-encroached drylands (MW, $p > 0.05$).

Soil C:N ratios, which range from 11 ±3 to 15 ±3, remain constant with changes in cover type in shrub-encroached drylands (KW, $p > 0.05$; Figure 2.4b). In contrast, we determined soil C:N ratios, which range from 10 ±4 to 16 ±4 vary with cover type in emerging ice-free arid ecosystems (KW, $p < 0.05$). Specifically, the C:N ratio of bare soil is lower than C:N ratios for non-vascular (DT, $p < 0.05$), herbaceous (DT, $p < 0.05$) and woody (DT, $p < 0.05$) soils. Further, soil C:N ratios for the herbaceous (MW, $p < 0.01$) and woody (MW, $p < 0.01$) cover types in emerging ice-free arid ecosystems are greater relative to the same cover types in shrub-encroached drylands.

The effect of cover type on the global soil NH_4^+-N pool varies between emerging ice-free ecosystems and shrub-encroached drylands (Figure 2.4c). Soil NH_4^+-N from emerging ice-free arid ecosystems decreases respectively from 1 ±1 μg g^{-1} to 0.3 ±0.2 μg g^{-1} between the bare and non-vascular cover types (DT, $p < 0.05$) before increasing to ~4 μg g^{-1} under the herbaceous and woody cover types (DT, $p < 0.001$). In contrast, NH_4^+-N in shrub-encroached drylands increases sharply from 3 ±1 μg g^{-1} to

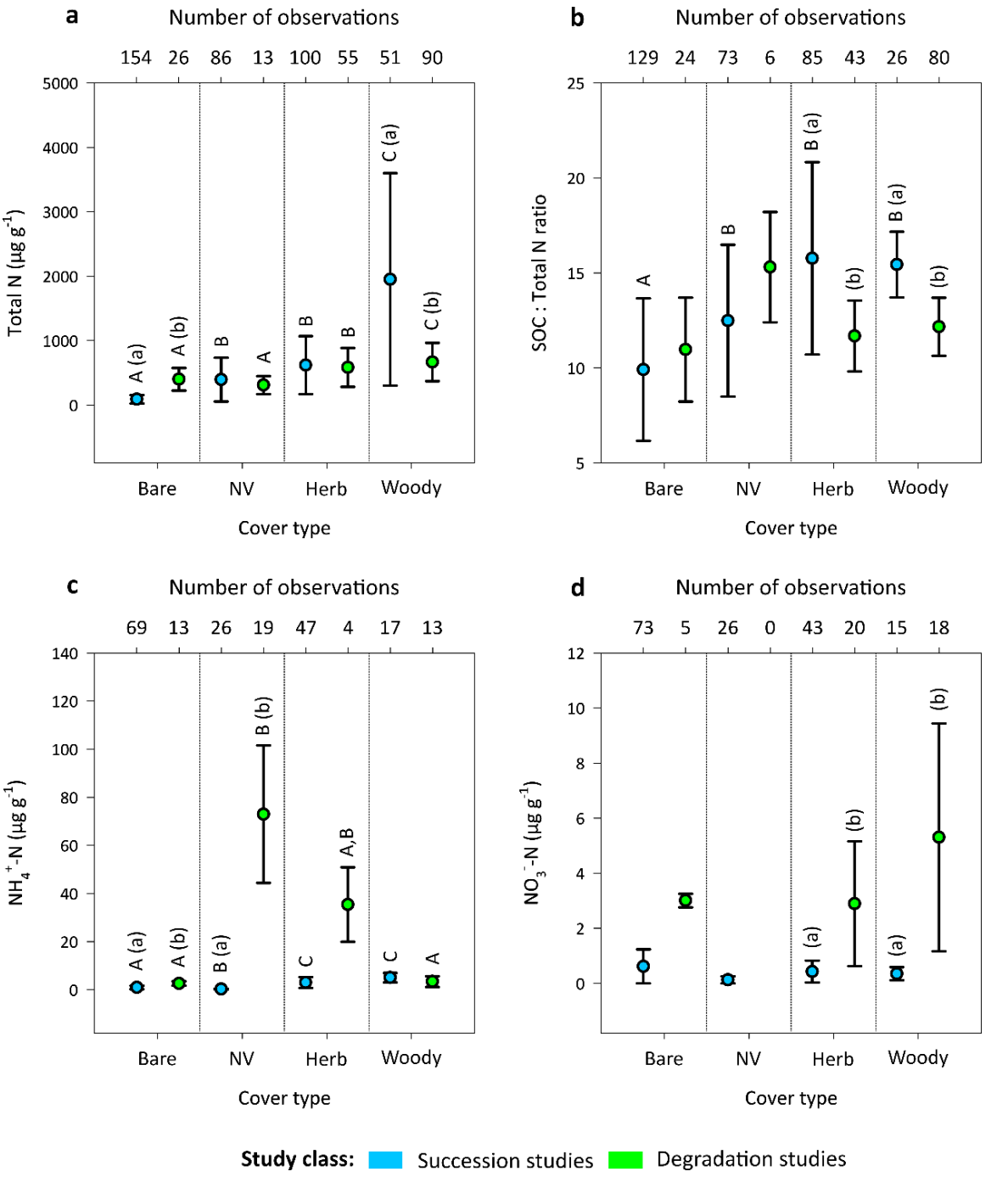

Figure 2.4 Median global total N contents (a), SOC:total N ratios (b), NH_4^+-N contents (c) and NO_3^--N contents (d) identified by meta-analysis for surface soils (top ≤15 cm) under bare soil (bare), non-vascular (NV), herbaceous (herb) and woody cover types in emerging ice-free (succession) and shrub-encroached (degradation) arid ecosystems derived for the following Köppen-Geiger climate classes (Peel *et al.*, 2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot desert steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). See tables 2.2 and 2.3 for source data and Appendix A for SOC data. Error bars represent one median absolute deviation about the median. Upper- and lower-case letters indicate significant differences at $p < 0.05$ within classes by cover type and between classes within cover types, respectively (Mann Whitney *U* test for two groups; Dunn's test for more than two groups; groups with n < 5 were excluded from tests of significance).

73 ±29 μg g^{-1} between the bare and non-vascular cover types (DT, $p < 0.001$), but decreases to3 ±2 μg g^{-1} under the woody cover type (DT, $p < 0.001$), where NH_4^+-N concentrations do not differ between bare and woody soils (DT, $p > 0.05$). The NH_4^+-N content of bare (MW, $p < 0.001$) and non-vascular (MW, $p < 0.05$) soils in shrub-encroached drylands are greater than the same cover types in emerging ice-free arid ecosystems.

We determined that the global NO_3^--N pool does not vary with cover type for either the emerging ice-free arid ecosystems or shrub-encroached drylands (Figure 2.4d). Median concentrations of NO_3^--N ranged from 0.1 ±0.1 μg g^{-1} to 1 ±1 μg g^{-1} in emerging ice-free arid ecosystems and 3 ±2 μg g^{-1} to 5 ±4 μg g^{-1} in shrub-encroached drylands. However, concentrations of NO_3^--N in soil were determined to be greater in shrub-encroached drylands relative to emerging ice-free arid ecosystems for both the herbaceous (MW, $p < 0.001$) and woody (MW, $p < 0.001$) cover types.

2.5 Discussion

Our study revealed that cover type exerted a greater control on soil N concentrations and C:N ratios in emerging ice-free arid ecosystems than shrub-encroached drylands. Further, the extent of aridity influenced the speciation of N (i.e., the amount of N in the inorganic phase) more strongly in emerging ice-free arid ecosystems than in shrub-encroached drylands. Here we discuss specifically how the N content, as well as C:N stoichiometry, of soil varies with cover type in emerging ice-free arid ecosystems and shrub-encroached drylands that have been delineated according to the Köppen-Geiger climate classification scheme (Peel *et al.*, 2007).

In nutrient-limited systems, it is necessary for pioneer species to enhance an environment to facilitate colonisation by later-successional species (see Figure 2.5 for an example of primary succession in a glacier forefield; Chapin *et al.*, 1994). Before herbaceous and, later, woody vegetation can colonise the surface of emerging ice-free arid ecosystems, bare soil must first be stabilised by non-vascular cover types, such as biological soil crusts (polyextremotolerant soil surface communities which contain N-fixing cyanobacteria and cyanolichens; Hodkinson *et al.*, 2003). Thus, if cover type classes in the order bare soil, non-vascular, herbaceous and woody are viewed as a linear trajectory of primary succession in emerging ice-free arid ecosystems, soil total N can be seen to increase globally with vegetation change between bare and woody cover types from 90 ±66 μg g^{-1} to 1,950 ±1,650 μg g^{-1} (Figure 2.4a). Increases in total N above the concentrations present in bare soil, which range from four- to 20-fold increases among the polar frost, polar tundra and cold arid steppe climate classes, generally occur in soils beneath herbaceous cover (Figure 2.2a). These increases likely arise from inputs of litter- and root-derived N to the soil system (Duc *et al.*, 2009) and support studies which determined that total N in soil increases significantly with the colonisation of the surface by herbaceous plants in emerging ice-free arid ecosystems (Chapin *et al.*, 1994; Hodkinson *et al.*, 2003; Turpin-Jelfs *et al.*, 2019b, Chapter 3

in this thesis). However, the total N content of non-vascular soil is higher than bare soil in the polar frost climate class. This result corroborates studies within this climate class which show that non-vascular assemblages, such as biological soil crusts, may also be capable of making significant contributions to the soil N pool in certain areas (Colesie *et al.*, 2014; Cowan *et al.*, 2011).

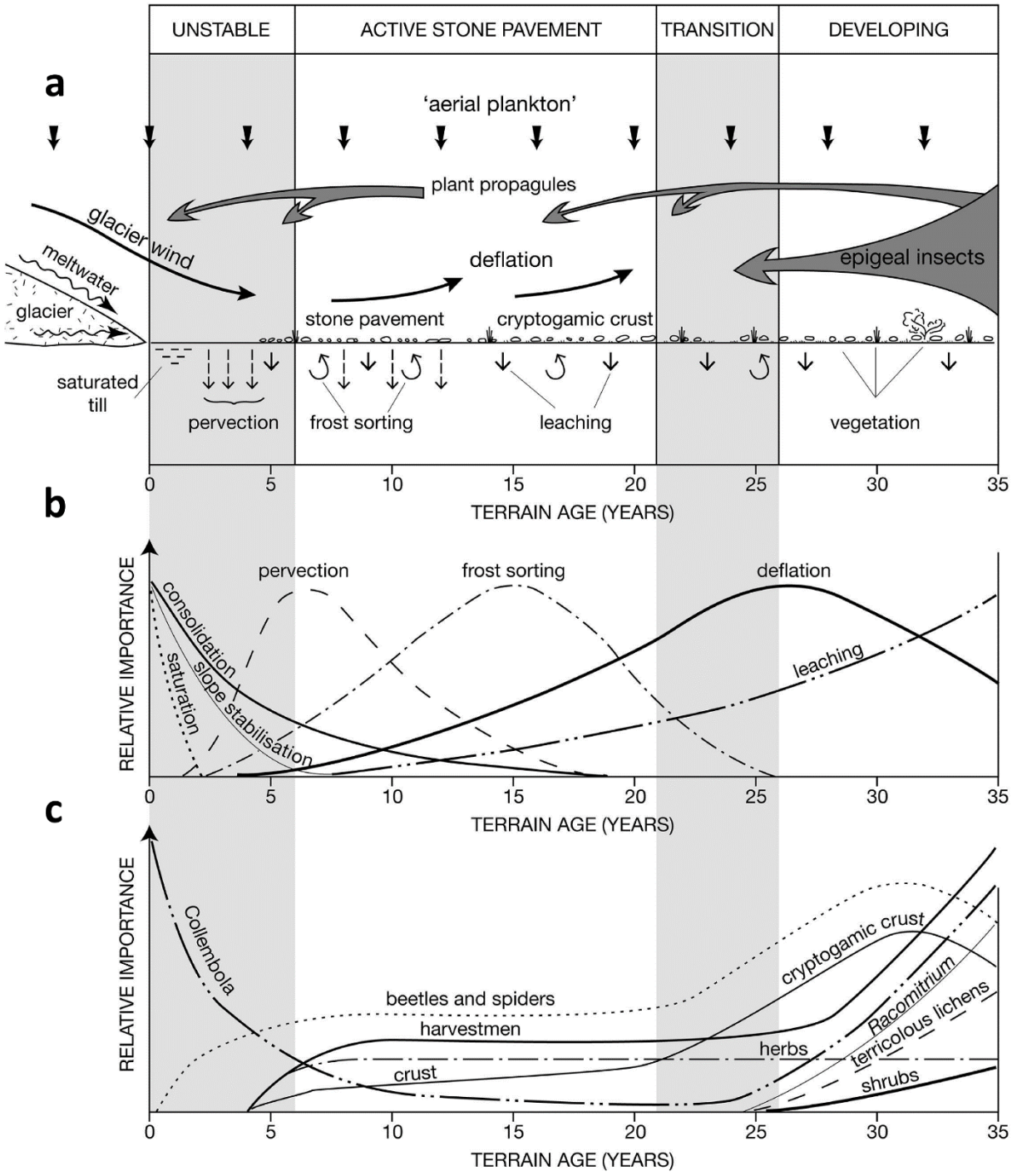

Figure 2.5 A conceptual model describing the interactions between biotic and abiotic ecosystem components (a), the relative importance of abiotic processes (b), and the importance plant and invertebrate groups (c) with geo-ecological succession in a glacier forefield. Columns denote the major phases of succession (image from Matthews and Vater, 2015).

As with emerging ice-free arid ecosystems, the total N content of shrub-encroached dryland soils increases globally between bare soil and woody cover types from 400 ±176 μg g^{-1} to 666 ±296 μg g^{-1} (Figure 2.4a). However, as woody plant encroachment is accompanied by an overall reduction in plant cover (e.g., Turnbull *et al.*, 2010), it is important that vegetation heterogeneity is considered when evaluating the effects of surface-cover changes on the total N pool in these systems. If the percentage of bare soil, herbaceous and woody cover identified for end-member sites along a gradient of woody plant encroachment (non-vascular cover not included as total N content does not differ from bare soil at $p < 0.05$) by Turpin-Jelfs *et al.* (2019a, Chapter 4 in this thesis) are considered, it is possible to roughly calculate changes in total N at the ecosystem level (g m^{-2}) using the equation (2.2):

$$Total\ N_{ecosystem} = bare\ soil\ cover\left(\frac{total\ N_{bare\ soil}}{10^6} \times \rho \times soil\ depth \times 10^5\right)$$

$$+ grass\ cover\left(\frac{total\ N_{herbaceous}}{10^6} \times \rho \times soil\ depth \times 10^5\right) \quad \textbf{(2.2)}$$

$$+ shrub\ cover\left(\frac{total\ N_{shrub}}{10^6} \times \rho \times soil\ depth \times 10^5\right)$$

where; ρ is bulk density (see Table 2.4 for data and units used in these calculations). These back-of-the-envelope calculations reveal a small decrease from 78 ±40 g m^{-2} to 73 ±32 g m^{-2} between grass- and shrub-dominants states. The similarity between these concentrations validate the findings of Turpin-Jelfs *et al.* (2019a, Chapter 4 in this thesis) which showed that at the ecosystem level, concentrations of total N remain constant with increasing woody cover, as declines of total N in intershrub areas (sum of bare soil, non-vascular and herbaceous cover) are balanced by gains of total N in soils beneath shrub canopies. Further, these results support the widely-held perception that grassland-to-shrubland transitions increase the spatial heterogeneity of soil N in dryland ecosystems by concentrating nutrients beneath shrub canopies (e.g., Schlesinger *et al.*, 1990; Turnbull *et al.*, 2010; Wheeler *et al.*, 2007). This redistribution of soil N will likely reduce herbaceous cover and promote the expansion of woody vegetation (Ravi *et al.*, 2010b).

Table 2.4 Percentages of vegetation cover, soil bulk density and soil depth used to estimate the ecosystem-level total N pool in shrub-encroached drylands with low and high levels of encroachment (data from Turpin-Jelfs *et al.*, 2019a, Chapter 4 in this thesis).

Encroachment level	Bare soil cover (%)	Herbaceous cover (%)	Shrub cover (%)	Bulk density (g cm^{-3})	Soil depth (cm)
Low	0	96	4	1.33	10
High	44	0	56	1.33	10

Similar to the findings of Turpin-Jelfs *et al.* (2019a, Chapter 4 in this thesis), >98 % of total N was found to be within the organic phase (total N minus the sum of NH_4^+-N and NO_3^--N) of soils from both emerging ice-free arid ecosystems and shrub-encroached drylands (Figure 2.4a, c, d), which suggests that N is quickly assimilated in these nutrient-limited ecosystems. The total N content of both bare and woody soils differ between emerging ice-free arid ecosystems and shrub-encroached drylands (Figure 2.4a). Specifically, concentrations of total N in bare soils were lower in emerging ice-free arid ecosystems relative to shrub-encroached drylands, whereas the total N contents of woody soils in emerging ice-free arid ecosystems were greater in comparison to shrub-encroached drylands. As the export of N associated with runoff and eroded-sediment yields has been shown to be lower in bare soils than soils under grasses in a dryland of the southwestern US (Michaelides *et al*., 2012), we argue the higher concentrations of total N in bare soils from shrub-encroached drylands relative to those from emerging ice-free arid ecosystems are due to a legacy of past vegetation cover. In contrast, as N mineralisation and moisture infiltration (which controls N leaching) rates share negative relationships with the soil clay content (Anderson, 1988; Virginia and Jarrell, 1983), it is possible that differences in the total N concentrations of woody soils between emerging ice-free arid ecosystems and shrub-encroached drylands are due to variations in soil texture. This is supported by studies which showed that whilst the soil clay fraction ranges from 50-80 % in emerging ice-free arid soils under vascular plants (Turpin-Jelfs *et al.*, 2019b, Chapter 3 in this thesis), it only ranges from 0.1-0.6 % in woody soils from shrub-encroached drylands (Turpin-Jelfs *et al.*, 2019a, Chapter 4 in this thesis). Despite lower concentrations of total N in bare soil, these results suggest that the potential for N storage in emerging ice-free arid ecosystems will ultimately surpass that of degrading shrub-encroached drylands with surface-cover change.

In addition to influencing N mineralisation and infiltration rates, increases in the soil clay content limit nitrification processes by providing a greater surface area for the adsorption of NH_4^+-N (Sahrawat, 2008). Thus, it may be argued that the lower concentrations of NO_3^--N in herbaceous and woody soils within emerging ice-free ecosystems relative to the same cover types within shrub-encroached drylands are to be expected due to differences in the soil clay content (Figure 2.4d). However, as concentrations of NH_4^+-N in woody soils do not differ between these arid environments (Figure 2.4c), it is perhaps more likely that differences in the availability of NO_3^--N are driven by the control of C:N ratios on decomposition processes (Schulten and Schnitzer, 1997), for which the C:N ratio of herbaceous and woody soils are greater in emerging ice-free arid ecosystems relative to shrub-encroached drylands (Figure 2.4b). This is supported by studies which have shown that rates of N mineralisation and nitrification in soil are inversely related to ratios of SOC to total N (Aulakh *et al.*, 2000; Ross *et al.*, 2004). Regardless of the factors responsible for creating these differences, the total N pool of herbaceous and woody soils in shrub-encroached drylands can be considered more

susceptible to losses than emerging ice-free arid ecosystems as NO_3^--N is highly mobile in soil (Rao and Puttanna, 2000).

As C:N ratios for bare and non-vascular soils do not vary between emerging ice-free arid ecosystems and shrub-encroached drylands (Figure 2.4b), differences in the availability of NH_4^+-N within these soils cannot confidently be ascribed to changes in the rate at which organic matter is mineralised (Figure 2.4c). When considering rates of NH_4^+-N leaching from biological soil crusts have been shown to vary with the assemblage of the community (Veluci *et al.*, 2006), it may be argued that concentrations of NH_4^+-N in non-vascular soils are higher in shrub-encroached drylands relative to emerging ice-free arid ecosystems as a result of differences between the compositions of this cover type. However, as our findings contradict a study by Castillo-Monroy *et al.* (2010) which showed that concentrations of NH_4^+-N do not differ between soils under bare and non-vascular cover types, it is possible that the NH_4^+-N levels for non-vascular soils in shrub-encroached drylands reported here may be uncharacteristically high. In contrast to non-vascular soils, it may be reasoned that concentrations of NH_4^+-N are lower in bare soils from emerging ice-free arid ecosystems relative to shrub-encroached drylands because the small total N pool in these systems encourages the rapid immobilisation of N by the microbial community (Bradley *et al.*, 2016). Indeed, concentrations of NH_4^+-N increase between bare and woody soils in emerging ice-free arid ecosystems coincident with gains in the total N pool (Figure 2.4a).

Since the biological processes which mediate nutrient cycles in arid ecosystems are driven by moisture availability (Nielsen and Ball, 2015), it would be reasonable to expect that changes in the extent of aridity would influence concentrations of C and N in the soil system. Yet we found no evidence to suggest that soil N and C:N ratios for shrub-encroached drylands vary among climate classes by cover type (Figure 2.3a-d). These findings contradict a study by Delgado-Baquerizo *et al.* (2013) which determined concentrations of SOC and total N decline in soil with increasing aridity due to reductions in plant cover and organic matter decomposition. Further, N losses resulting from decreased plant cover were argued to increase soil C:N ratios. In contrast to shrub-encroached drylands, we did identify variations among climate classes for soil C:N ratios (Figure 2.2b), as well as NO_3^--N concentrations (Figure 2.2d), under both bare and non-vascular cover types in emerging ice-free arid ecosystems. Hence, whilst identifying a direct cause for these differences is challenging, it does suggest that emerging ice-free arid ecosystems are more susceptible to climate perturbations than shrub-encroached drylands.

Caution is recommended when interpreting the results presented here, as the high degree of variability evident within the data (figures 2.2-2.4) could potentially obscure trends or differences among the N contents and C:N stoichiometries of soils along and between trajectories of succession and

degradation (Petrie and Sabine, 2009). This variability is likely a consequence of differences in the mineralogy of geologic parent material, for which the quality and quantity of organic matter stored in soil is controlled by aggregate stability, weathering products, and the percentage clay fraction (Deiss *et al.*, 2018). Further, mineralogical differences in parent material can affect soil pH which is important for determining the extent and rate of microbiological nutrient transformations, where soil pH in the range 4.3-7.4 was determined to be positively related with microbial catabolic rates (Wakelin *et al.*, 2008). As vegetation change has been argued to be driven by the strength of plant-soil feedbacks (Schlesinger and Pilmanis, 1998), it is important that further research considers the reciprocal influences of edaphic properties and cover type on the distribution and speciation of C and N in emerging ice-free and shrub-encroached arid ecosystems.

2.6 Conclusion

Climate warming and shifts in land use over the past ca. 150 years have promoted surface-cover changes in high latitude and altitude ecosystems and water-limited grasslands, respectively. Using meta-analysis, we determined that the total N pool of soils exposed by declining ice and snow cover increases between bare soil and woody cover types. Further, if cover type change in the order bare soil, non-vascular, herbaceous and woody is viewed as a linear trajectory of primary succession in these emerging ice-free arid ecosystems, our findings show that significant gains in soil total N generally occur with the colonisation of the surface by vascular plants. Like emerging ice-free arid ecosystems, the total N content of shrub-encroached grassland soil increases between bare soil and woody cover types. Yet if differences in total N are weighted according to concurrent changes in surface cover, we found the ecosystem-level total N pool remains constant with grassland-to-shrubland transitions but is concentrated into soils beneath shrub canopies. This redistribution of N will likely favour the persistence of woody plants and result in a reduction of herbaceous cover. When the total N pool in soils beneath cover types were compared between emerging ice-free and shrub-encroached arid ecosystems, we found that the largest pool of mineralisable N occurred beneath woody cover in emerging ice-free arid ecosystems. This suggests that emerging ice-free arid ecosystems will ultimately become more productive than shrub-encroached drylands with continued soil development and primary succession. However, differences in soil C:N ratios among the varying degrees of aridity for bare soil and non-vascular cover types in ice-free arid ecosystems suggest soil development in high latitude and altitude regions may be sensitive to climate perturbations. When considering ice-free and shrub-encroached areas are projected to expand, this study has implications for understanding how surface-cover changes will modify ecosystem productivity in arid regions.

2.7 Acknowledgments

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3 Distribution of soil nitrogen and nitrogenase activity in the forefield of a High Arctic receding glacier

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Turpin-Jelfs, T.,[4] Michaelides, K.,[1,5] Blacker, J.J.,[6] Benning, L.G.,[3,7,8] Williams, J.M.[1] and Anesio, A.M.[1,9] (2019). Distribution of soil nitrogen and nitrogenase activity in the forefield of a High Arctic receding glacier. *Annals of Glaciology*, 59(77): 87-94. doi:10.1017/aog.2018.35

The lead author, Thomas Turpin-Jelfs, led the design of the study, conducted all sample (excluding phosphorus analysis) and data analysis, and wrote the manuscript. Co-author Joshua J. Blacker provided phosphorus data for all sequentially fractionated soil samples. Co-author James M. Williams provided technical assistance with inorganic nitrogen extractions. Co-authors Katerina Michaelides, Liane G. Benning and Alexandre M. Anesio advised on the methodology and edited the manuscript.

Soil samples were collected in accordance with the methods outlined in section 3.3.2 by Katherine Wright[1] and James A. Bradley[1] on behalf of Alexandre M. Anesio in 2013 prior to the start of this research project.

The article explores how the distribution and speciation of nitrogen, as well as rates of free-living biological nitrogen fixation, change along a 2,000-year chronosequence of soil development in a High Arctic glacier forefield within the polar tundra (ET) Köppen-Geiger climate class (Peel *et al.*, 2007), and addresses the second objective of this thesis (see section 1.2).

All data presented in this chapter are available online (Turpin-Jelfs and Michaelides, 2018b).

[4] School of Geographical Sciences, University of Bristol, Bristol, BS8 1SS, UK

[5] Earth Research Institute, University of California Santa Barbara, Santa Barbara, CA, US

[6] School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, UK

[7] GFZ, German Research Centre for Geosciences, 14473, Potsdam, Germany

[8] Department of Earth Sciences, Free University of Berlin, 12249, Berlin, Germany

[9] Department of Environmental Science, Aarhus University, 4000 Roskilde, Denmark

3.1 Abstract

Glaciers retreating in response to climate warming are progressively exposing primary mineral substrates to surface conditions. As primary production is constrained by nitrogen availability in these emerging ecosystems, improving our understanding of how nitrogen accumulates with soil formation is of critical concern. In this study, we quantified how the distribution and speciation of nitrogen, as well as rates of free-living biological nitrogen fixation (BNF), change along a 2,000-year chronosequence of soil development in a High Arctic glacier forefield. Our results show the soil nitrogen pool increases with time since exposure and that the rate at which it accumulates is influenced by soil texture. Further, all nitrogen increases were organically bound in soils which had been ice-free for 0-50 years. This is indicative of nitrogen limitation and should promote BNF. Using the acetylene reduction assay technique, we demonstrated that microbially-mediated inputs of nitrogen only occurred in soils that had been exposed for ≤3 years, and that potential rates of BNF declined with increased nitrogen availability. Thus, BNF only supports nitrogen accumulation in young soils. When considering that glacier forefields are projected to become more expansive, this study has implications for understanding how ice-free ecosystems will become productive over time.

3.2 Introduction

Since ca. 1850, glacier coverage in high latitude and altitude regions has declined in response to climate warming (Stocker *et al.*, 2013). Retreating glaciers progressively expose soil-forming mineral substrates to surface conditions and biotic colonisation (Breen and Lévesque, 2008). Thus, glacier forefields are comprised from linear arrays of soil development stages which provide unique opportunities to investigate long-term primary succession and ecosystem development (Bradley *et al.*, 2014). As terrestrial primary production and ecosystem functioning are typically limited by the availability of nitrogen (N; Vitousek *et al.*, 1997a), advancing our understanding of how N accumulates with soil formation is crucial to identifying the long-term productivity of these ecosystems (Bradley *et al.*, 2014).

The development of soils at high latitudes occurs over relatively long timescales as soil forming processes are highly constrained by low temperatures, short growing seasons and slow weathering rates (Ellis and Mellor, 1995). Using space-for-time substitutions, where distance from the leading edge of a retreating glacier serves as a proxy for soil age (post-incisive chronosequence), it has been shown that soil N typically increases with time since glacial retreat (Bradley *et al.*, 2014). Principal inputs which contribute to the accumulation of N in these ecosystems include biological N fixation (BNF) by free-living (asymbiotic) soil bacteria and plant-microbe associations, mineralisation of organic matter previously overridden by the ice, and allochthonous loadings from aerial deposition and glacial runoff (Brankatschk *et al.*, 2011). Whilst the relative contributions of these inputs may vary, asymbiotic BNF

is widely considered to be the dominant source of assimilatory N during the initial stages of soil development due to the low nutrient status of recently exposed soils (Bradley *et al.*, 2014).

As primary mineral substrates generally lack N (Vitousek and Farrington, 1997), the initial colonisation of recently deglaciated soils is largely restricted to asymbiotic microorganisms with the capacity to convert atmospheric dinitrogen (N_2) into N-containing organic compounds (diazotrophs; Duc *et al.*, 2009). This process is mediated by the nitrogenase enzyme which catalases the adenosine triphosphate-dependent reduction of N_2 to ammonia (Vitousek *et al.*, 2002b). Due to a high phosphorus (P) demand for adenosine triphosphate, it is expected that increases in the availability of soil N relative to bioavailable P will be suppress BNF processes (Menge and Hedin, 2009). However, the factors influencing the extent and distribution of BNF in glacier forefield ecosystems are poorly resolved (Brankatschk *et al.*, 2011).

Inputs of N by asymbiotic BNF to forefield systems facilitate subsequent colonisation by heterotrophic microorganisms capable of mineralising soil organic matter (Bradley *et al.*, 2014). As soil organic matter typically contains >90 % of all soil N, mineralisation represents a major process controlling the availability of N in many terrestrial ecosystems. Inorganic N, which is defined hereinafter as the sum of ammonium (NH_4^+-N) and nitrate (NO_3^--N), is either liberated or immobilised by the heterotrophic community during mineralisation when N is in excess or limiting, respectively (Hassink, 1994). Thus, the availability of inorganic N is strongly influenced by the stoichiometry between soil organic carbon (SOC) and total N (Delgado-Baquerizo *et al.*, 2015). Whilst previous studies have quantified inorganic N availability in forefield soils (e.g., Duc *et al.*, 2009; Knelman *et al.*, 2012; Töwe *et al.*, 2010), knowledge concerning the development of this pool over periods exceeding ca. 150 years is limited.

The main aim of this study was to assess how time on soil development since glacial retreat in a High Arctic glacier forefield impacts the soil N status and inputs of N by asymbiotic BNF along a 2,000-year chronosequence.

3.3 Materials and methods

3.3.1 Study site description

The study was conducted in the forefield of the Midtre Lovénbreen alpine-type polythermal valley-glacier (Figure 3.1; 78˚55’ N, 12˚10’ E) in northwest Spitsbergen, Svalbard, Norway, at an altitude of ~50 m asl. Comprising an area of ~5.5 km^2, the glacier is 1 km wide at its equilibrium line (~365 m asl), exhibits a maximum thickness of 180 m and rises in elevation over 6 km from 50 m asl at the terminus to 600 m asl at the head (Rippin *et al.*, 2003). The glacier has retreated by ~1 km since reaching its Neoglacial maximum extent during the early twentieth century, causing predominantly coarse-grained (5-20 cm) mineral soil of metamorphic parent material to be exposed to atmospheric conditions

(Moreau *et al.*, 2008; Rippin *et al.*, 2003). Vegetation cover in the forefield is sparse, where *Saxifraga oppositifolia* L., a vascular plant common to Arctic regions, is the most dominant species at each site along the chronosequence (for detailed information on vegetation cover see Hodkinson *et al.*, 2003). The mean annual temperature for the years 1981 to 2010 was -5.2 °C. Mean annual precipitation for the same period was 427 mm (Førland *et al.*, 2011). The average 1-cm soil temperature during the summer growing season (early July to late August) was 8 °C (Bekku *et al.*, 2003).

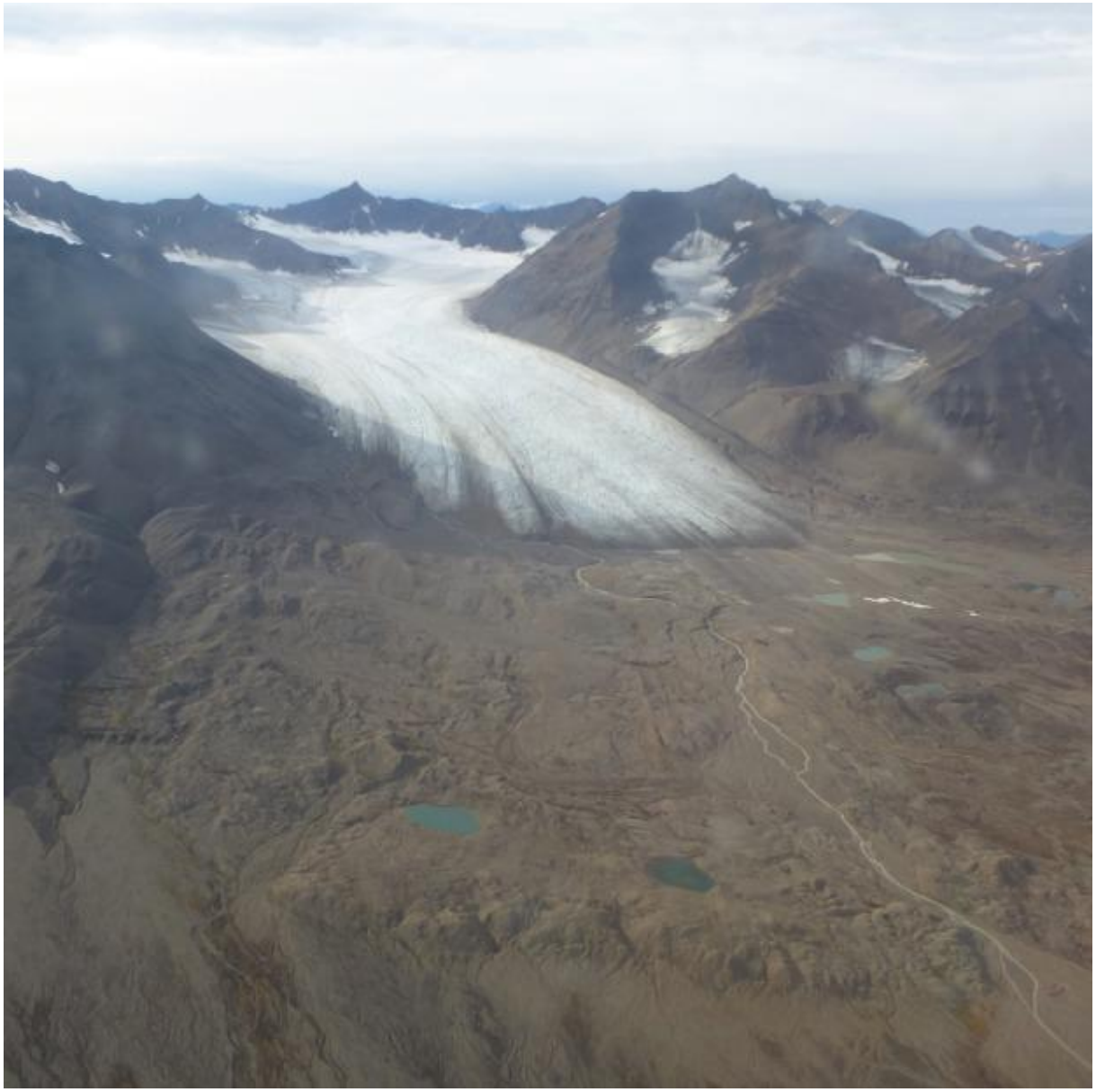

Figure 3.1 Aerial photograph of the Midtre Lovénbreen alpine-type polythermal valley-glacier and forefield in Svalbard, Norway (78˚55' N, 12˚10' E). Facing south-southwest. To approximate scale, the proglacial lakes range from 40-100 m in diameter (image from Bradley *et al.*, 2014).

3.3.2 Sample collection and preparation

Surface soil (top 10 cm) samples were collected along a proglacial post-incisive chronosequence between July and August 2013 (Bradley *et al.*, 2016). The chronosequence was comprised of three parallel transects which were installed perpendicular to the leading edge of the glacier (Figure 3.2). At each sampling site along the three transects, a 10-m traverse was installed parallel to the glacier's leading edge, where soil samples were extracted at 0 m, 5 m and 10 m across its length. Sampling sites occur at irregular intervals corresponding to the following soil ages (time since glacial retreat): 0, 3, 5, 29 and 50 years (n = 9). In addition, one of the three transects included 113-year-old and >1,900 (2,000 hereinafter)-year-old sampling sites (n = 3). Soil ages had previously been determined by Bradley *et al.* (2016) and Hodkinson *et al.* (2003) using satellite imagery (Landsat TM 7), photographic records and carbon (C)-14 dating techniques. As described by Bradley *et al.* (2016), the fine earth fraction (≤2,000 µm) of the soil samples was separated from coarser material in the field, transferred to high-density polyethylene bags (Whirl-Pak®; Lactun, Australia) and immediately frozen at -20 °C. Representative aliquots of frozen samples were freeze-dried for elemental analysis, whereas aliquots for the determination of soil texture, soil nutrient concentrations and nitrogenase activity were thawed in the dark at 4 °C prior to analysis.

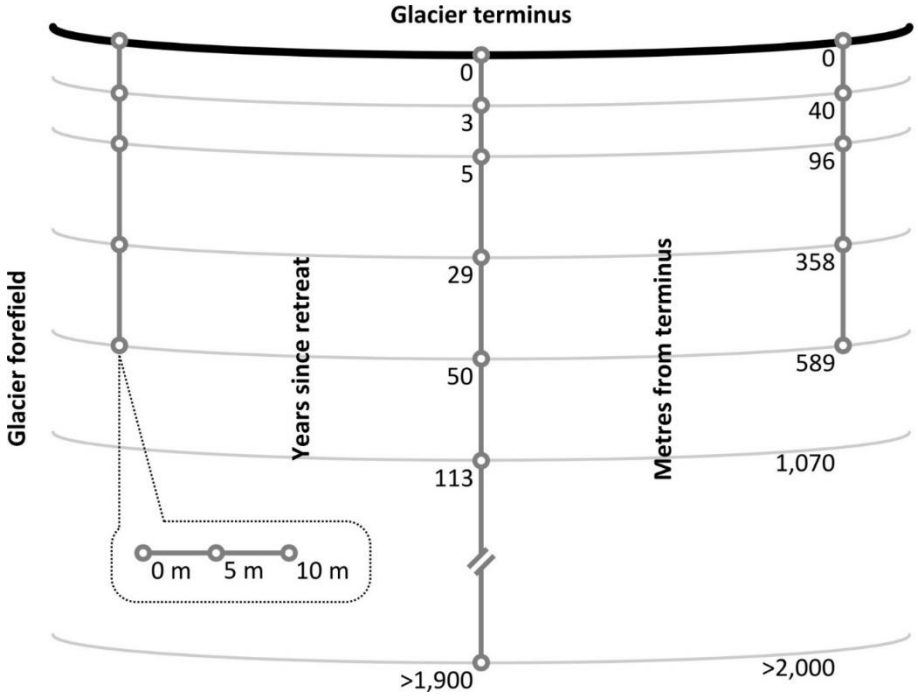

Figure 3.2 Plan view of three transects which had been installed ~127 m apart between centres perpendicular to the terminus of a glacier to systematically sample the surface soil (10 cm) of a glacier forefield at irregular intervals corresponding to soil age (not to scale). Three soil samples were collected at 0-, 5- and 10-m intervals along a traverse parallel to the terminus at each sampling point (n = 9 for 0- to 50-year-old soils; n = 3 for 113- to >1,900-year-old soils).

3.3.3 Soil texture

A Malvern Mastersizer 3000 laser particle size analyser (Malvern Panalytical, Worcestershire, UK) in the measurement range of 0.02 µm to 2 mm was used to determine the particle-size distribution of the soil fine earth fraction, where samples were dispersed in a solution of sodium hexametaphosphate and sodium carbonate using hydro EV pump accessory (Lamorski *et al.*, 2014). Soil texture was subsequently defined according to the USDA-SCS (1982) classification scheme, and the percentage sand and clay content used to estimate water holding capacity (WHC; %; Saxton and Rawls, 2006).

3.3.4 Soil organic carbon and total nitrogen

An elemental analyser (CHNS-O EA 1108 Elemental Analyzer; Carlo Erba, Milan, Italy) was used to determine the SOC and total N content in ~100 mg aliquots of soil after inorganic C had been removed by HCl vaporisation according to methods described by Hedges and Stern (1984). Prior to analysis, freeze-dried samples were embrittled using liquid N_2, and ground to a flour using a pestle and mortar. The detection limits were 100 µg g^{-1} for both elements measured and the coefficient of variation for C and N according to six replicates of an organic analytical standard (NC Soil Standard 338 40025, cert. 133317, C = 2.29 %, N = 0.21 %; Elemental Microanalysis Ltd., UK) were ±1.69 % and ±1.36 %, respectively. Due to the limited amount of sample available, soil organic matter was in turn estimated from SOC using a conversion factor of 2, which is based on the assumption that 50 % of organic matter is comprised of C (Pribyl, 2010).

3.3.5 Soil nutrient analyses

Concentrations of exchangeable NH_4^+-N and NO_3^--N were determined by KCl extraction where aliquots of 0.2 g were amended with 10 mL of 2M KCl (1:50 w/v soil:extractant), shaken for 30 minutes at 160 rpm, centrifuged for 5 minutes at 4,500 rpm and filtered to 0.45 µm using Whatman WCN plain cellulose nitrate filtrate papers. Concentrations of NH_4^+-N (QuikChem® method 31-107-06-1-I) and NO_3^--N (QuikChem® method 31-107-04-1-K) within the resulting extracts were quantified colorimetrically using a flow injection analyser (Lachat QuikChem 8500 Series 2 FIA system, Loveland, CO, US), where the coefficient of variation (six replicate standards) for NH_4^+-N and NO_3^--N was ±3.7 % and ±0.9 %, respectively according to mid-range standards (calibration range: 0-0.5 mg L^{-1}). The respective detection limits (three times the standard deviation of six replicate method blanks) for NH_4^+-N and NO_3^--N were 0.04 µg g^{-1} and 0.01 µg g^{-1} for dry sediment. Bioavailable P (sum of loosely-sorbed, and iron- and aluminium-bound P) was sequentially extracted from 0.2 g of soil using the SEDEX method (Ruttenberg, 1992; Ruttenberg *et al.*, 2009) and analysed colorimetrically using a segmented flow analyser (SEAL AA3, Norderstedt, Germany). Detection limits (three times the standard deviation of six replicate method blanks) for both loosely-sorbed, and iron- and aluminium-bound P was 0.05 µg g^{-1}. The coefficient of variation (six replicate standards) for the same fractions

were both <0.5 %. Samples were blank-corrected when blank concentrations exceeded the detection limits. All results were reported on a dry weight basis, where percentage dry matter content (DMC) was determined gravimetrically using methods described by Rowell (1994).

3.3.6 Biological nitrogen fixation

Nitrogenase activity was assessed using the acetylene (C_2H_2) reduction assay technique adapted from Telling *et al.* (2011) as described by Turpin-Jelfs *et al.* (2018). The number of samples per soil age was reduced from nine to three by homogenising the three samples obtained from the 10-m traverses at each sampling site along the transects (n = 3). Aliquots from each sample (equivalent to 7.5 g dry weight) were transferred to gas-tight 30 mL serum bottles and incubated at 8 ±0.1 ˚C (mean summer temperature) under 16 W florescent lamps (Sylvania, Garching, Germany) with emission spectra in the range of 300-700 nm and irradiance equal to 0.2 ±0.005 W m^{-2}. Following a 72-hour pre-incubation period, 10 % of the headspace from each bottle was replaced with 100 % C_2H_2 gas, which had been produced by adding Milli-Q water to technical grade calcium carbide (Sigma, St. Louis, MO, US). Serum bottles were sampled using a gas-tight syringe at 0-, 3-, 6-, 12-, 20- and 24-hour intervals. At the time of sampling, 5 mL of air:C_2H_2 (9:1) was added to each serum bottle, mixed thoroughly by plunging the syringe six times and subsequently transferred to pre-evacuated 3.7 mL Exetainers (Labco, Lampeter, UK). A 1 mL aliquot of headspace from each Exetainer was then analysed using a Varian 3800 gas chromatograph (GC; Varian, Inc., Palo Alto, CA, US), where ethylene (C_2H_4) was separated from C_2H_2 using a 6’ x 1/8”, 80/100 mesh HayeSep T column at 85 ˚C (helium carrier gas). Daily standards of 100 ppm C_2H_4 (BOC, Guildford, UK) gave precisions of <5 %. The precision for 100 ppm standards that had been stored in 3.7 mL for a period of one month also gave precisions of <5 %. Detection limits were <0.01 nmol C_2H_4 g^{-1} for dry sediment. The C_2H_4 produced in 24 hours was converted to N_2 using the theoretical molar ratio of 3:1 (BNF_{day}; nmol N g^{-1} d^{-1}). Potential annual rates of asymbiotic BNF per unit area (BNF_{annual}; g N m^{-2} a^{-1}) were determined using the equation (3.1):

$$BNF_{annual} = BNF_{day} \times depth_{soil} \times \rho \times time \quad \textbf{(3.1)}$$

where; $depth_{soil}$ was the top 1 cm of the surface soil, ρ was the reference bulk density (g cm^{-3}), which was calculated as a function of the soil clay, silt, sand and organic matter content (Keller and Håkansson, 2010), and time was the length of the summer melt season (60 days; Hodson *et al.*, 2005). All sample measurements were blank-corrected using corresponding autoclaved soil samples (126 ˚C for 30 minutes). Controls for natural C_2H_4 production in the absence of C_2H_2 and C_2H_4 consumption were consistently below the limits of detection. The method was validated using nodulated white clover (*Trifolium repens* L.) root systems, which contain known N-fixing *Rhizobium* spp. (Francis and Alexander, 1972), prior to use in this study (see Appendix B).

3.3.7 Statistical analysis

Statistical analysis of the data was performed using R version 3.5.0 in accordance with principles outlined by Crawley (2005). The Kruskal-Wallis *H* test (KW) was used to determine if significant differences occurred for data with n > 3 between soil ages along the forefield chronosequence. When significance was indicated, Dunn's test (DT) of multiple comparisons was applied. Relationships between explanatory and response variables were explored using simple linear regression analysis, where residuals were inspected for evidence of non-normality using the Shapiro-Wilk test. To reduce leverage by extreme predictors, data pertaining to the 2,000-year-old soil samples were not included in regression models. For this study, the alpha level was set to 0.05. All errors reported in the text are one median absolute deviation about the median.

3.4 Results

3.4.1 Soil texture

The 0- to 50-year-old soils were silt loams, whereas the 113- to 2,000-year-old soils were clays (Figure 3.3). Soil clay content was significantly influenced by soil age (DT, $p < 0.001$), where clay particles decrease from 6 ±1 % to 4 ±1 % between the 0- to 50-year-old soils (DT, $p < 0.001$). The soil clay content then increases dramatically to 50 ±17 % and 80 ±3 % in the 113- and 2,000-year old soils, respectively. Soil silt and sand fractions do not differ between the 0- to 50-year-old soils ($p > 0.05$). However, the silt fraction decreases from 64 ±1 % in the 50-year-old soil to ~12 % in the 113- and 2,000-year-old soils. Further, the soil sand content decreases sharply from 38 ±12 % in the 113-year-old soil to 10 ±1 % in the 2,000-year-old soil.

3.4.2 Soil carbon and nitrogen

The SOC content of the forefield soil increases with soil age ($R^2 = 0.97$, $p < 0.01$; see Appendix C for regression analysis plots). Concentrations of SOC increase from ~530 ±90 μg g^{-1} in the 0- and 3-year-old soils to ~840 ±290 μg g^{-1} in the 5- to 50-year-old soils (DT, $p < 0.05$; Figure 3.4a). Subsequent to this, concentrations of SOC increase to 3,093 ±1,705 μg g^{-1} in the 113-year-old soils and 60,800 ±11,550 μg g^{-1} in the 2,000-year-old soils.

Like SOC, the total N content of the forefield soils is strongly influenced by soil age ($R^2 = 0.92$, $p < 0.01$). Between the 0- and 50-year-old soils, concentrations of total N increase from 84 ±12 μg g^{-1} to 215 ±47 μg g^{-1} (Figure 3.4b). Specifically, the total N content of the 0-year-old soil is lower in comparison with the 3- to 50-year-old soils (DT, $p < 0.05$), and the total N content of the 3-year-old soil is lower relative to that of the 29- and 50-year-old soils (DT, $p < 0.05$). In addition, concentrations of total N subsequently rise to 606 ±70 μg g^{-1} in the 113-year-old soil and to 4,400 ±500 μg g^{-1} in the 2,000-year-old soil.

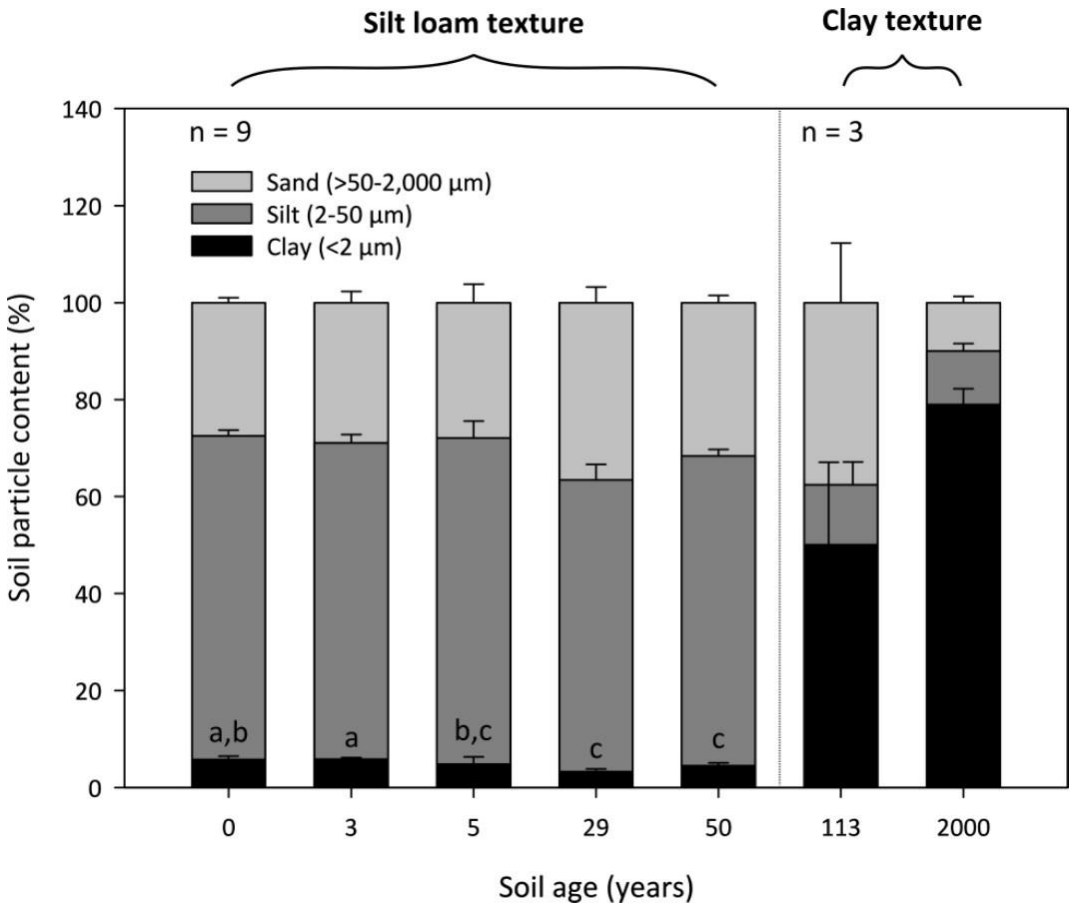

Figure 3.3 Median percentage clay, silt and sand content of surface soil (top 10 cm) from a glacier forefield in Svalbard, Norway. Groups denoted with different letters indicate significant differences at $p < 0.05$ (Dunn's test). Error bars represent median absolute deviation about the median. Soil textural classes are displayed in bold and were defined according to the USDA-SCS (1982) classification scheme.

The relative contribution of inorganic N to total N decreases from 4 % in the 0-year-old soil to 1 % in the 2,000-year-old soil. Inorganic N within the 0- to 50-year-old soils is chiefly comprised of NO_3^--N, where median concentrations of NH_4^+-N are below the limits of detection (Figure 3.4c, d). Further, NO_3^--N levels are not influenced by soil age and do not differ between the 0- to 50-year-old soils (KW, $p > 0.05$). Conversely, the inorganic N content of the 113-year-old soil increases by 55 % above that of the 50-year-old soil. Subsequent gains of both NH_4^+-N and NO_3^--N within the 2,000-year-old soil serve to increase inorganic N by 586 % above the concentrations observed in the 113-year-old soil, of which only 21 % is in the form of NO_3^--N.

3.4.3 Nutrient ratios

Ratios of SOC:total N (C:N) are not significantly influenced by soil age ($R^2 = 0.11$, $p > 0.05$) and remain constant along the encroachment gradient where the median C:N ratios in the 0- and 113-year-old soils are 8 ±3 and 7 ±2, respectively (Figure 3.5a). However, inorganic N:bioavailable P (N:P) ratios

increase linearly with soil age (R^2 = 0.77, p < 0.05) from 0.3 ±0.1 in the 0-year-old soil to 0.6 ±0.1 in the 113-year-old soil (Figure 3.5b). Further, the N:P ratios of the 0- to 113-year-old soils generally exhibited lower variability than corresponding C:N ratios.

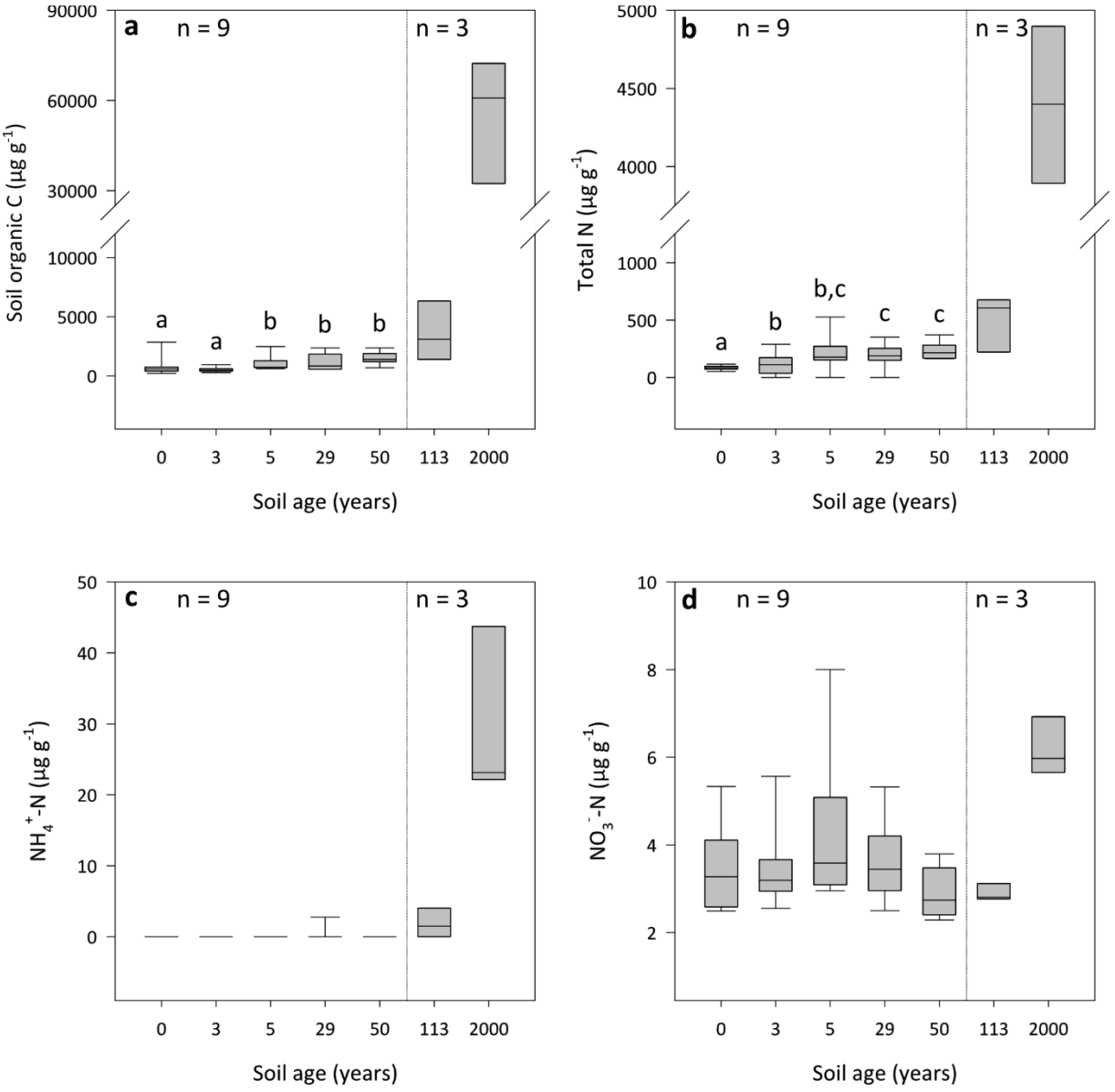

Figure 3.4 Soil organic (a), total N (b), NH_4^+-N (c) and NO_3^--N (d) concentrations for surface soil (top 10 cm) by dry weight for a glacier forefield chronosequence in Svalbard, Norway. Boxes denoted with different letters indicate significant differences at p < 0.05 (Dunn's test). Results reported on dry matter basis.

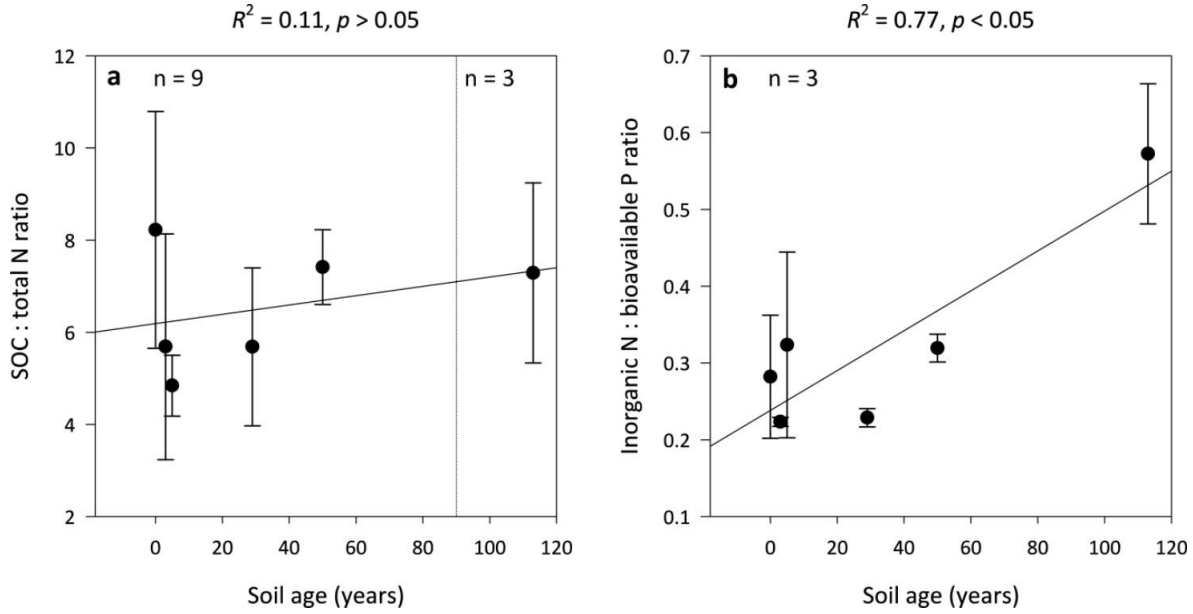

Figure 3.5 Median SOC:total N (a) and inorganic N:bioavailable P (b) ratios for surface soil (top 10 cm) for a glacier forefield chronosequence in Svalbard, Norway. Error bars represent median absolute deviation about the median. Results reported on dry matter basis.

3.4.4 Acetylene reduction assay

Potential annual rates of asymbiotic BNF decrease with soil age from 0.16 ±0.04 g N m^{-2} a^{-1} in the 0-year-old soil to below detection limits in the 5-year-old soil. Potential BNF rates were derived from rates of C_2H_4 production, which were linear over the 24-hour period for both the 0- (R^2 = 0.95, p < 0.01) and 3-year-old (R^2 = 0.67, p < 0.05) soils (Figure 3.6a-c). C_2H_2 reduction assays of the 29-, 50-, 113- and 2,000-year-old soils were not performed as C_2H_4 production during the preliminary incubations of these samples, as well as the 5-year-old samples, were below the limits of detection (data not shown here). The preliminary incubations, which were also performed in triplicate, adhered to the methods previously outlined (i.e., incubated in the light at 8 ˚C), but were only sampled at 0- and 24-hour intervals after the 72-hour pre-incubation period. These preliminary incubations were also corrected for background C_2H_4 levels via blanks containing autoclaved soil samples in the presence of C_2H_2 and assessed for natural C_2H_4 production and consumption.

3.5 Discussion

Our study revealed that time on soil development since glacial retreat strongly influences the distribution and speciation of N in the surface soil of the Midtre Lovénbreen glacier forefield. Further, we determined that N inputs by asymbiotic diazotrophs represent an important source of bioavailable N to this ecosystem. Here we discuss specifically how the soil N pool, as well as potential rates of asymbiotic BNF, vary along a 2,000-year chronosequence of soil development in a High Arctic glacier forefield.

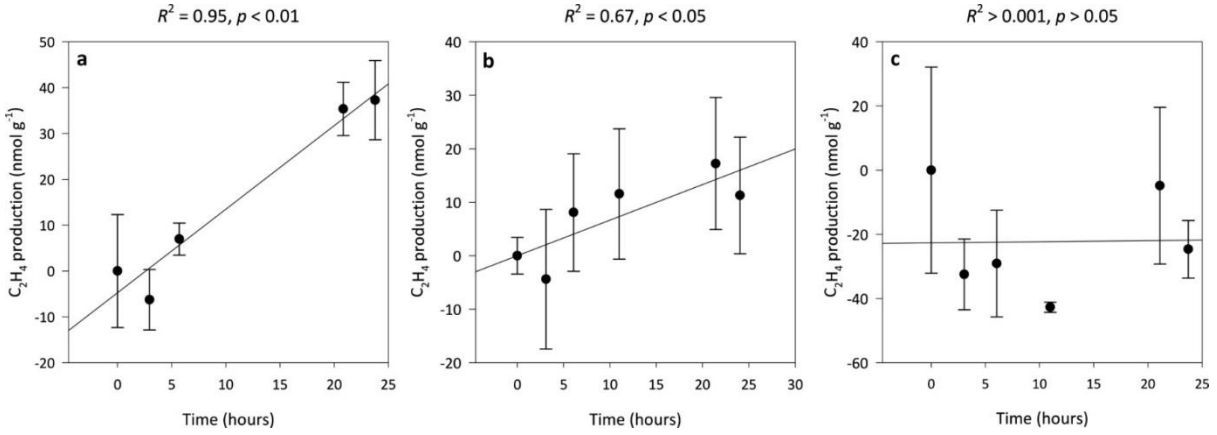

Figure 3.6 Median ethylene (C_2H_4) production for 0- (a), 3- (b) and 5-year-old (c) soils from a glacier forefield in Svalbard in Svalbard, Norway. Error bars represent median absolute deviation about the median (n = 3). Results reported on dry matter basis.

3.5.1 Distribution and speciation of soil nitrogen

Our findings are in agreement with a comprehensive review carried out by Bradley *et al.* (2014) which showed the total N content of glacier forefield soils typically increase with soil age (Figure 3.4b). We observed that total N initially increases by ~100 µg g^{-1} above 84 ±12 µg g^{-1} between the 0- and 50-year-old soils, which are dominated by a silt loam texture (Figure 3.3). The accumulation of total N subsequently accelerates in clay textured soils which have been ice-free for 113 (606 ±70 µg g^{-1}) and 2,000 (4,399 ±498 µg g^{-1}) years, and which were determined by Hodkinson *et al.* (2003) to exhibit significantly higher vascular plant cover than soils that have been exposed for <100 years (see Appendix D for changes in the percentage of surface cover with soil age by plant taxa). Greater concentrations of total N in vegetated soils are to be expected as plants have been shown to increase the soil N pool through inputs of nutrient-rich litter and exudates, as well symbiotically fixed N resulting from associations with rhizobia (Duc *et al.*, 2009). In addition, the soil clay content, which increases substantially between the silt loam- and clay-textured soils, is important for stabilising soil organic matter (Egli *et al.*, 2010), where the percentage of total N which is organically bound (total N minus the sum of NH_4^+-N and NO_3^--N) ranges from 96 ±1 % in the 0-year-old soil to 99 ±0.1 % in the 2,000-year-old soil (Figure 3.4b-d). Interestingly, increases in total N between the 0- and 50-year-old soils did not share a clear relationship with the soil clay content. This discovery is corroborated by a study of the Damma glacier forefield, Switzerland, by Dümig *et al.* (2011) which also failed to identify a clear trend in the soil clay content with age, and demonstrated that the accumulation of soil organic matter did not share a linear relationship with soil texture. Incidentally, whilst soil textural changes driven by cryogenic and aeolian processes may be rapid in glacier forefields (Matthews, 1992), the large unsampled interval between the 50- and 113-year-old soils makes it challenging to establish when the rate of clay accumulation increased.

In contrast to model simulations by Bradley *et al.* (2016) which showed that inorganic N accumulates at a relatively constant rate within Midtre Lovénbreen forefield, we observed that concentrations of inorganic N remain largely stable at 3-4 µg g^{-1} between the 0- and 113-year-old soils (Figure 3.4c, d). As NO_3^--N was found to constitute 100 % of inorganic N in the silt loam-textured soils (ice-free for 0 to 50 years), it may be reasoned that inputs of NH_4^+-N are rapidly immobilised by the soil microbial biomass (Bradley *et al.*, 2016). Indeed, a study of the Loch Vale Watershed in the Colorado Rocky Mountains, US, by Baron *et al.* (1995) showed that NH_4^+-N, which comprised ~50 % of received inorganic N deposition, was incorporated directly into biomass or oxidised to NO_3^--N in soil, as well as snow pack and surface waters. Conversely, the increase in NH_4^+-N in the 113- and 2,000-year-old soils (Figure 3.4c) suggests that mineralisation processes are sufficient to facilitate the accumulation of inorganic N in the clay-textured soils (Bradley *et al.*, 2016). This is supported by studies of the Damma glacier (Switzerland; Töwe *et al.*, 2010), and Ödenwinkelkees and Rotmoosferner glaciers (Austria; Tscherko *et al.*, 2003) which showed that functional genes (*chiA* and *aprA*) and rates of N mineralisation increase with soil age, respectively. Despite soil C:N ratios remaining constant across the chronosequence (Figure 3.5a), the lack of inorganic N accumulation in the silt loam-textured soils is indicative of N limitation (Schimel *et al.*, 2004). Consequently, the capacity for BNF should represent a selective advantage in the silt loam-textured soils.

It should be noted that is possible we may have underestimated the SOC and total N content of the forefield soils. Despite the widespread use of standard protocols which exclude the coarse particle fraction (>2,000 µm) from SOC and total N analyses (e.g., Bernasconi *et al.*, 2011; Dümig *et al.*, 2011; Throop and Archer, 2008; Turnbull *et al.*, 2010; Wheeler *et al.*, 2007), studies have shown that coarser grained materials may accumulate significant amounts of C and N with pedogenesis (e.g., Agnelli *et al.*, 2001; Corti *et al.*, 2002; Cunliffe *et al.*, 2016b). However, as a study by Corti *et al.* (2002) showed the total C and N contained in the coarse particle fraction of soils (0-50 cm depth) derived from metamorphic parent material was 0.5-0.9 % and 0 %, respectively, we argue that the exclusion of this fraction from our analyses would not have impacted significantly upon the data presented here. In addition, we recognise the space-for-time substitution (ergodic hypothesis) employed during this study as a potential constraint because it is sensitive to localised environmental conditions, such as variable allochthonous inputs of C and N, which are common to glacier forefields (Bradley *et al.*, 2014), that may influence between- and within-site variation (Walker *et al.*, 2010). Thus, whilst this method is useful for examining how High Arctic glacier forefield soils are likely to develop over time, it will be necessary to validate the results from this study using a repeated sampling approach before they may be extrapolated to the wider region.

3.5.2 Inputs of asymbiotically fixed nitrogen

We found that linear rates of asymbiotic BNF (C_2H_4 production) only occur at levels above the limits of detection in the 0- and 3-year-old soils from the Midtre Lovénbreen glacier forefield (Figure 3.6a-c). These rates of BNF measured in the forefield soils are more than five and ten times greater than those for marine terrace soils ~10 km from the study site (Solheim *et al.*, 1996) and the Midtre Lovénbreen glacier (Telling *et al.*, 2011), respectively, but are in the range reported for soils in polar desert and alpine tundra ecosystems by Cleveland *et al.* (1999), as well as cyanobacteria-bryophyte symbioses in Svalbard (Solheim *et al.*, 1996). Further, if, to facilitate comparisons with Telling *et al.* (2011), the 0- and 3-year-old soils were each conservatively assumed to represent 1 % of the Midtre Lovénbreen catchment area, they would collectively provide the system with ~2 kg N km^{-2} a^{-1}. This is two orders of magnitude greater than N inputs by the cryoconite microbial community on the glacier (Telling *et al.*, 2011), and equivalent to 5 % of annual N inputs to the catchment by rain and snow during the 1999/2000 melt season (Hodson *et al.*, 2005). Thus, despite constituting an overall lower input of N to the system than rain and snow, our findings suggest the microbially-mediated fixation of N by asymbiotic diazotrophs may provide an important source of assimilatory N during the initial stages of soil development in the forefield system. This is supported by studies for the Anvers Island (Antarctica; Strauss *et al.*, 2012), Damma (Switzerland; Töwe *et al.*, 2010), Puca (Peru; Schmidt *et al.*, 2008) and Mendenhall (AK, US; Knelman *et al.*, 2012; Sattin *et al.*, 2009) glaciers which showed that asymbiotic BNF promoted the accumulation of N and successional changes within the microbial community of recently deglaciated soils. However, in contrast to our findings, these studies have shown that nitrogenase activity typically increased in soils between 0 and 10 years of age (Sattin *et al.*, 2009; Schmidt *et al.*, 2008; Strauss *et al.*, 2012).

Nitrogenase activity declined with soil age in the Midtre Lovénbreen forefield (Figure 3.6a-c). As N:P ratios increase linearly with time on soil development (Figure 3.5b), these findings support the theory that nitrogenase activity will down-regulate as the availability of N increases relative to P (Smith, 1992), and are substantiated by Telling *et al.* (2011) who demonstrated that potential rates of asymbiotic BNF by the cryoconite microbial community on the Midtre Lovénbreen glacier, as well as the neighbouring Austre Brøggerbreen and Vestre Brøggerbreen glaciers, decline with increases in the availability of N. Further, a study by Nash *et al.* (2018) in the same site as ours showed the abundance of the *nifH* gene, an important marker of diazotrophs, increase as concentrations of total N decline below 1,000 μg g^{-1}. In contrast, Brankatschk *et al.* (2011) found the *nifH* gene abundance of soils from the Damma glacier forefield in Göscheneralp, Switzerland, increased with gains in total N between soils which had been ice-free 10 and 50 years. However, this increase was attributed to a greater competition for N as plant cover rose from <10 % to >70 % with the number of years since exposure. As gains in soil N in this study

were limited to the organic phase in the silt loam-textured soils, it may be inferred that increases in organic N with soil age are adequate to sustain growth within the microbial biomass.

We caution treating the potential BNF rates presented here as absolutes. Uniform incubation parameters were used to achieve linear rates of C_2H_2 reduction over a 24-hour period, where parameters were chosen to reflect mean summer growth/melt season conditions. As the soil microbial community in the Midtre Lovénbreen forefield is dominated by autotrophic organisms (Bradley *et al.*, 2017; Hodkinson *et al.*, 2003; Nash *et al.*, 2018), it was reasoned that nitrogenase activity, which is positively correlated with the soil moisture content (Chapin *et al.*, 1991), would be restricted to a period when water is available to the biota (Logan, 1968). However, despite low temporal variability in the soil N pool during the short summer season in Svalbard (Bardgett *et al.*, 2007), it may be argued that N inputs released to the soil solution through snowmelt and rainfall may influence potential rates of asymbiotic BNF. Indeed, Telling *et al.* (2011) demonstrated that N fixation by the cryoconite microbial community on the Midtre Lovénbreen glacier was constrained by inputs of N from snowmelt. For this reason, we consider it prudent to present potential rates of BNF as a range based on a conservative 20- and maximum 60-day summer period equal to 0.7-2 kg N km^{-2} a^{-1}. The lower extremum of this range is similar to potential rates of BNF by cryoconite on the sediment-covered marginal zone on Leverett Glacier in Greenland (0.8 kg N km^{-2} a^{-1}) which was determined to be important to the colonisation of moraine-derived sediments in that area (Telling *et al.*, 2012).

In addition to time, other factors may have introduced uncertainty into the BNF data presented here. As the depth to which autotrophs can colonise is limited by the availability of photosynthetically active radiation (Jeffery *et al.*, 2009), our estimates of fixation per unit area were restricted to the top 1 cm of the surface soil, as light penetration beyond this depth in bare and coarse soils would not be significant (Tester and Morris, 1987). Such an assumption may be insufficient for adequately characterising contributions of N fixation by heterotrophic diazotrophs, which may occur throughout the surface soil matrix and achieve comparable rates of BNF to their autotrophic counterparts (Strauss *et al.*, 2012). Further, as photosynthesis, which shares a positive relationship with nitrogenase activity, increases with higher irradiance (Rabouille *et al.*, 2006), it is possible that potential rates of BNF were limited by the incubation light source in the laboratory experiments being three orders of magnitude lower than the global average of 198 W m^{-2} (Le Treut *et al.*, 2007). Lastly, as C_2H_4:N_2 conversion factors for naturally-occurring cyanobacteria in soil range from 1.9 to 6.1, it is recommended that conversions of C_2H_4 to N are calibrated using a ^{15}N dilution (Belnap, 2001). As conversions from C_2H_4 to N were not calibrated in this study, it is possible that we may have over- or under-estimated potential rates of BNF in this system. Nevertheless, the data serve to illustrate that asymbiotic BNF is an important N input pathway to the Midtre Lovénbreen glacier forefield which declines with time on soil development (i.e., with the accumulation of N).

3.6 Conclusions

Over the past century, glaciers in Arctic regions have undergone significant retreat with rapid changes in climate, exposing new soil-forming substrates to atmospheric conditions. Using a space-for-time substitution approach, we showed that the soil N pool, which is essential to terrestrial primary production, increases with time since exposure in the forefield of a High Arctic glacier. However, the extent to which soil N increases with time is influenced by soil texture (i.e., percentage of clay content), where N accumulates more rapidly in clay-textured (113 and 2,000 years old) than silt loam-textured soils (0 to 50 years old). Further, in contrast to the clay-textured soils, we observed that gains in soil N were limited to the organic phase in the silt loam-textured soils. This suggests that inputs of inorganic N to the silt loam-textured soils are immobilised by the microbial biomass due to N-limiting conditions. Whilst N limitation should promote BNF, asymbiotic nitrogenase activity was only detectable in the 0- and 3-year-old soils, where potential annual rates of N fixation decline with increases in soil N. Nevertheless, we demonstrated that inputs of N by asymbiotic diazotrophs may represent an important source of assimilatory N during the initial stages of soil development in this glacier forefield. As glacier coverage is projected to decrease with continued climate warming, this study has implications for understanding how ice-free ecosystems develop and become productive over time.

3.7 Acknowledgements

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4 Soil nitrogen response to shrub encroachment in a degrading semi-arid grassland

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Turpin-Jelfs, T.,[1] Michaelides, K.,[1,2] Biederman, J.A.[3] and Anesio, A.M.[1,4] 2018. Soil nitrogen response to shrub encroachment in a degrading semiarid grassland. *Biogeosciences Discuss*. doi: https://doi.org/10.5194/bg-2018-331

The lead author, Thomas Turpin-Jelfs, led the design of the study, conducted all fieldwork, sample and data analysis, and manuscript writing. Co-authors Katerina Michaelides, Joel A. Biederman and Alexandre M. Anesio advised on the methodology and edited the manuscript. It should be noted that sections 4.3.5, 4.3.6 and 4.3.8 have been abridged to prevent the unnecessary repetition of information presented elsewhere in this thesis.

The article explores how the distribution and speciation of soil nitrogen, as well as rates of free-living biological nitrogen fixation, change along a gradient of increasing mesquite (*Prosopis velutina* Woot.) cover in an arid grassland of the southwestern US within the hot arid steppe (BSh) Köppen-Geiger climate class (Peel *et al.*, 2007), and addresses the third objective of this thesis (see section 1.2).

Please note that for consistency with previously published literature, the term “semi-arid” is used in place of “hot arid steppe” in this chapter.

All data presented in this chapter are available online (Turpin-Jelfs and Michaelides, 2018a).

[1] School of Geographical Sciences, University of Bristol, Bristol, BS8 1SS, UK

[2] Earth Research Institute, University of California Santa Barbara, Santa Barbara, CA, US

[3] Southwest Watershed Research Center, United States Agricultural Department, Tucson, AZ, US

[4] Department of Environmental Science, Aarhus University, 4000 Roskilde, Denmark

4.1 Abstract

Transitions from grass- to shrub-dominated states in drylands by woody plant encroachment represent significant forms of land cover change with the potential to alter the spatial distribution and cycling of soil resources. Yet an understanding of how this phenomenon impacts the soil nitrogen pool, which is essential to primary production in arid and semi-arid systems, is poorly resolved. In this study, we quantified how the distribution and speciation of soil nitrogen, as well as rates of free-living biological nitrogen fixation, changed along a gradient of increasing mesquite (*Prosopis velutina* Woot.) cover in a semi-arid grassland of the southwestern US. Our results show that site-level concentrations of total nitrogen remain unchanged at 86 ±22 g m^{-2} with increasing shrub cover because losses from intershrub areas (sum of grass and bare-soil cover) are balanced by increases in soils under shrub canopies. However, despite the similar carbon-to-nitrogen ratio (~8) and microbial biomass (~2 nmol g^{-1}) of soil from intershrub and shrub areas at each site, site-level (0.25 ha) concentrations of inorganic nitrogen increase from 0.3 ±0.2 g m^{-2} to 0.8 ±0.5 g m^{-2} with shrub cover due to the accumulation of ammonium and nitrate in soils beneath shrub canopies. Using the acetylene reduction assay technique, we found increasing ratios of inorganic nitrogen to bioavailable phosphorus inhibit rates of biological nitrogen fixation by free-living soil bacteria. Overall, these results provide a greater insight into how grassland-to-shrubland transitions influence the soil N pool through associated impacts on the soil microbial biomass.

4.2 Introduction

Degrading dryland landscapes undergo significant ecological transformations typically associated with gradual and irreversible changes in plant community composition (D'Odorico *et al.*, 2013). Woody plant encroachment is a widespread example of dryland degradation, whereby continuous grasslands are progressively replaced by shrubs interspersed by patches of bare soil (Ravi *et al.*, 2010a). These plant community changes result in a structurally, physically and biologically different ecosystem which significantly alters the spatial distribution and fluxes of nutrients and the biogeochemical cycling within dryland landscapes, with implications for the ongoing process of land degradation (Eldridge *et al.*, 2011; Michaelides *et al.*, 2012). Nitrogen (N), in particular, is considered the most significant limiting nutrient of primary production and decomposition processes in arid and semi-arid ecosystems (Gebauer and Ehleringer, 2000), yet there is currently limited understanding of how ecosystem transformations associated with land degradation change the distribution, speciation and cycling of N (Browning *et al.*, 2008), as well as the associated soil microbial biomass (Li *et al.*, 2017). As drylands occupy ~41 % of Earth's land surface, changes in the nutrient distributions, patterns and cycling within degrading areas have important implications for biogeochemical cycles at the global scale (Delgado-Baquerizo *et al.*, 2013).

Occurring in Africa (Hudak *et al.*, 2003), Asia (Zhang *et al.*, 2016b), Australia (Cookson *et al.*, 2006), Europe (Quero *et al.*, 2013), North America (Knapp *et al.*, 2008) and South America (Cabral *et al.*, 2003), extensive research has demonstrated that shrub encroachment into grasslands alters the scale of vegetation heterogeneity within the landscape from fine to large scales and changes the microtopography from relatively subdued to high relief as soil mounds develop beneath shrub canopies (Charley and West, 1975; Schlesinger *et al.*, 1996; Wainwright *et al.*, 2000). These changes in both vegetation heterogeneity and microtopography result in modifications to runoff and erosion patterns (Michaelides *et al.*, 2009; Parsons *et al.*, 1996), which directly affect the redistribution of, and patterns in, nutrients within the landscape in favour of shrub functional types (Cross and Schlesinger, 1999; Michaelides *et al.*, 2012; Schlesinger *et al.*, 1996; Turnbull *et al.*, 2010). In addition, shrubs may further concentrate nutrients beneath their canopies through increased inputs of high-quality above- and below-ground detritus (Schlesinger *et al.*, 1990; Throop and Archer, 2008). The resulting accumulation of soil organic matter (SOM) in shrub mounds represents an important pool of mineralisable N that provides shrubs with a competitive advantage over herbaceous plants during times of nutrient limitation (Turnbull *et al.*, 2010).

In terrestrial systems, the availability of inorganic N, defined hereafter as the sum of ammonium (NH_4^+-N) and nitrate (NO_3^--N), is largely controlled by microbial decomposition processes (Vitousek *et al.*, 2002b). The dominant decomposers in soil are bacteria and fungi, of which fungi typically exhibit greater carbon (C):N ratios than bacteria, as well as enhanced C use efficiency and reduced rates of nutrient turnover (Waring *et al.*, 2013). As the amount of N mineralised during decomposition depends on the C and N stoichiometry of decomposer organisms relative to that of organic matter (Schulten and Schnitzer, 1997), differences in the relative abundance of fungal and bacterial biomass can potentially modulate soil N cycling (Waring *et al.*, 2013). Using the phospholipid fatty acid (PLFA) method, Li *et al.* (2017) demonstrated that the ratio of viable fungi to bacteria for a semi-arid grassland was significantly higher in soil under shrubs than for soils in intershrub areas, and that soil microbial biomass was positively correlated with concentrations of total N and available phosphorus (P). However, an understanding of how the fungal and bacterial constituents of the microbial biomass change along a gradient of increasing shrub cover within a semi-arid grassland is currently lacking.

Whilst mineralisation may control the availability of N, biological N fixation (BNF) by free-living (asymbiotic) soil bacteria and legume-*Rhizobium* symbioses is the principal input of reactive N to semi-arid systems (Evans and Ehleringer, 1993), in which rates of symbiotic BNF are approximately an order of magnitude higher than inputs through asymbiotic pathways (Cleveland *et al.*, 1999). Many encroaching shrub species, such as mesquite (*Prosopis* spp.), are legumes capable of symbiotic BNF, which reduces their dependence on soil N mineralisation and offers the potential to increase the availability of N within a system (Blaser *et al.*, 2014). However, since BNF has a high P demand, rates

of symbiotic dinitrogen (N_2) fixation by mesquite have been shown to down-regulate as N increases relative to concentrations of available P (Geesing *et al.*, 2000). Less studied are the effects of ecosystem transformations on inputs of asymbiotically fixed N to semi-arid grasslands, which despite being lower than contributions by symbiotic BNF, are still significant due to the small size of the soil N pool in these systems.

The aim of this study was to assess how a transition from a grass- to shrub-dominated state within a dryland ecosystem impacts the soil N status and inputs of N by asymbiotic BNF. Specifically, our objectives were

1. to examine how pools of total N, NH_4^+-N and NO_3^--N, as well as the microbial biomass, in soil varied across a shrub encroachment gradient; and
2. to determine how the distribution and supply rates of asymbiotically fixed N varied across a shrub encroachment gradient.

We expected that reductions in grass biomass resulting from higher levels of shrub encroachment would cause soil N to be progressively redistributed from intershrub zones to areas beneath shrub canopies. As the spatial distribution of soil microorganisms is positively influenced by resource availability (Schlesinger and Pilmanis, 1998), we hypothesised the enhanced fertility and environmental conditions in soils under shrubs would support a higher soil microbial biomass and promote N mineralisation processes, leading to an increased availability of inorganic N. Accordingly, we predicted that rates of asymbiotic BNF would decrease in shrub soils as inorganic N increased relative to bioavailable P (Smith, 1992).

4.3 Materials and methods

4.3.1 Study site description

Fieldwork was conducted between March and April 2016 at the Santa Rita Experimental Range (SRER; Figure 4.1a; 31˚54’ N, 110˚53’ W), Arizona, in the southwestern US, a region where the density of mesquite has increased by >400 % over the past ca. 30 years alone (Resco de Dios *et al.*, 2012). Situated on a bajada comprising ~21,000 ha, the SRER ranges in elevation from 900 m asl to 1,400 m asl (McClaran, 2003). Mean daily maximum and minimum air temperatures for the years 2008 to 2015 were 25 ˚C and 13 ˚C, respectively. Mean annual precipitation for the same period was 392 mm, with 61 % received during the summer months (July through September). The respective mean daily maximum and minimum summer temperatures between 2008 and 2015 were 31 ˚C and 20 ˚C (AmeriFlux, http://ameriflux.lbl.gov/data/download-data/). Vegetation is dominated by native desert grassland species, such as Rothrock grama (*Bouteloua rothrockii* Vasey), sideoats grama (*B. curtipendula* (Michx.) Torr.), Arizona cottontop (*Digitaria californica* (Benth.) Henrard), bush muhly

(*Muhlenbergia porteri* Scribn.) and tanglehead (*Heteropogon contortus* (L.) Beauv.), as well as the non-native Lehmann love grass (*Eragrostis lehmanniana* Nees.). Cacti, such as cholla cactus species (*Opuntia spp*. Mill.), and shrubs, such as velvet mesquite (*Prosopis velutina* Woot.) and catclaw acacia (*Acacia greggii* A. Gray), were also present (McClaran *et al.*, 2002).

Encroachment by mesquite within the SRER has been well documented (McClaran, 2003). Velvet mesquite, which was restricted to drainage arroyos in 1903, currently occupies ~35 % of the total grassland area (Figure 4.2) and represents the dominant shrub species within the elevation band of 990-1,200 m asl (Browning *et al.*, 2008). The following five sampling sites comprise a gradient of increasing shrub cover representing progressive ecosystem degradation: S-4, S-28, S-44, S-55 and S-56 (Figure 4.1b) where the number signifies the percentage of shrub cover. The percentage of shrub canopy and intershrub area was calculated for the five sites using high-resolution satellite imagery from Google Earth (2016a-e), which was cropped to the same dimensions and segmented using the Trainable Weka Segmentation plug-in for Fiji (http://fiji.sc/Fiji). Sobel and Gaussian blur filters were used for edge detection and noise suppression, respectively (Arganda-Carreras *et al.*, 2017). The out-of-bag error for all sites classified was <2 %. Soils at these sites were formed in alluvium from igneous rock of Holocene and Late Pleistocene origins (Batchily *et al.*, 2003) in the Combate-Diaspar complex (CoB), characterised by excellent drainage, sandy loam textures and 1-8 % slopes (SRER Digital Database, https://cals.arizona.edu/srer/data.html). All five sites were situated at ~1,250 m asl, exhibited similar topography, and had the same land use history.

4.3.2 Field measurements and sample collection

To mitigate the effects of directional bias during sampling, a 180-m transect (horizontal distance) was arranged in a Y-configuration at each site, where three 60-m transects radiated out from plot centres at azimuths of 30°, 150° and 270° (Figure 4.3). Three 100-m^2 quadrats were then installed at 10-, 30-, and 50-m intervals along each transect for the determination of above-ground shrub biomass. Further, a 0.5 m^2 quadrat was centred within the largest intershrub area of each 100 m^2 quadrat for the determination of above-ground grass biomass. The dry weight of each velvet mesquite plant was estimated from stem basal diameter measurements using methods and a site- and species-specific allometric equation reported in McClaran *et al.* (2013). Similarly, the dry weight of individual bunch grasses was estimated from tussock basal diameter measurements using methods, and a site-specific multispecies allometric equation reported in Nafus *et al.* (2009). The sums of the individual shrub and herbaceous constituents were calculated and used to estimate the total mesquite and grass biomass by dry weight for each site, where grass biomass was weighted according to intershrub area (kg m^{-2}). Finally, three intershrub and shrub surface soil (top 10 cm) cores were extracted at equal distances between the stem and drip line on the north side of the mesquite plant with the largest stem basal diameter and along the centre of the 0.5 m^2 quadrat, respectively. The extracted cores were then

homogenised to create single intershrub and shrub samples for each quadrat per site (n = 9) and sealed in sterile bags (Whirl-Pak®, Lactun, Australia).

4.3.3 Soil preparation

The mechanical sieving method described by Loveland and Whalley (2000) was used to evaluate the coarse fraction (>2,000 µm, wt %) of each sample. The resulting fine earth fraction (≤2,000 µm) from each sample was retained for all further analyses. For pH, PLFA, and elemental analyses, the number of intershrub and shrub samples per site was reduced to three (n = 3) by homogenising the samples collected along each of the three 60-m transects. Samples were then stored in a dark temperature-controlled environment at 4 ˚C. Prior to storage, aliquots of 5 g from each of the n = 9 and n = 3 samples were frozen at -20 ˚C and freeze-dried for elemental and PLFA analyses, respectively.

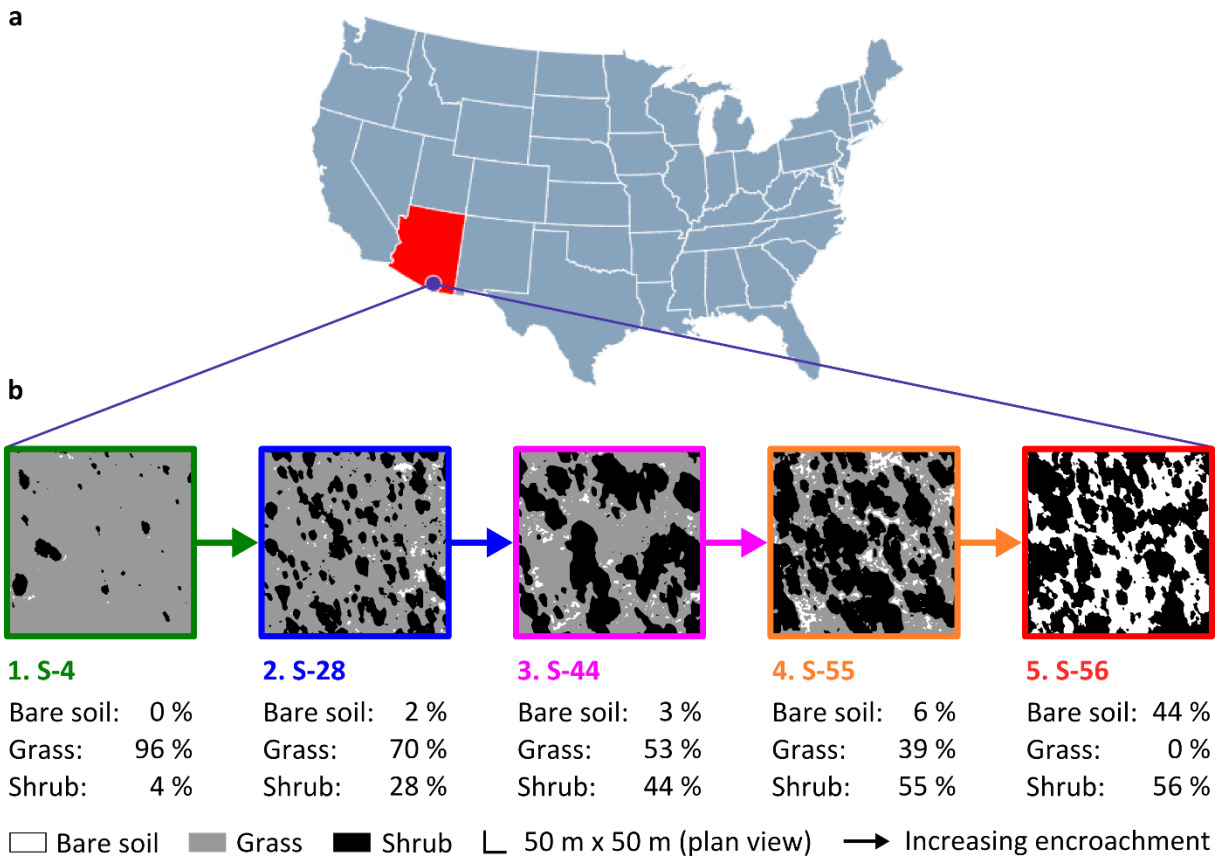

Figure 4.1 The study site (a) was a semi-arid grassland in the Santa Rita Experimental Range, AZ, US (31˚54' N, 110˚53' W; elevation: ~1,250 m asl). Five sampling sites comprised a gradient of shrub encroachment, where grass cover decreased, and bare soil and shrub cover increased between sites 1 and 5 (b). The intershrub area of each site is equal to the sum of the percentage of bare soil and grass cover. See Appendix E for ground-level site images.

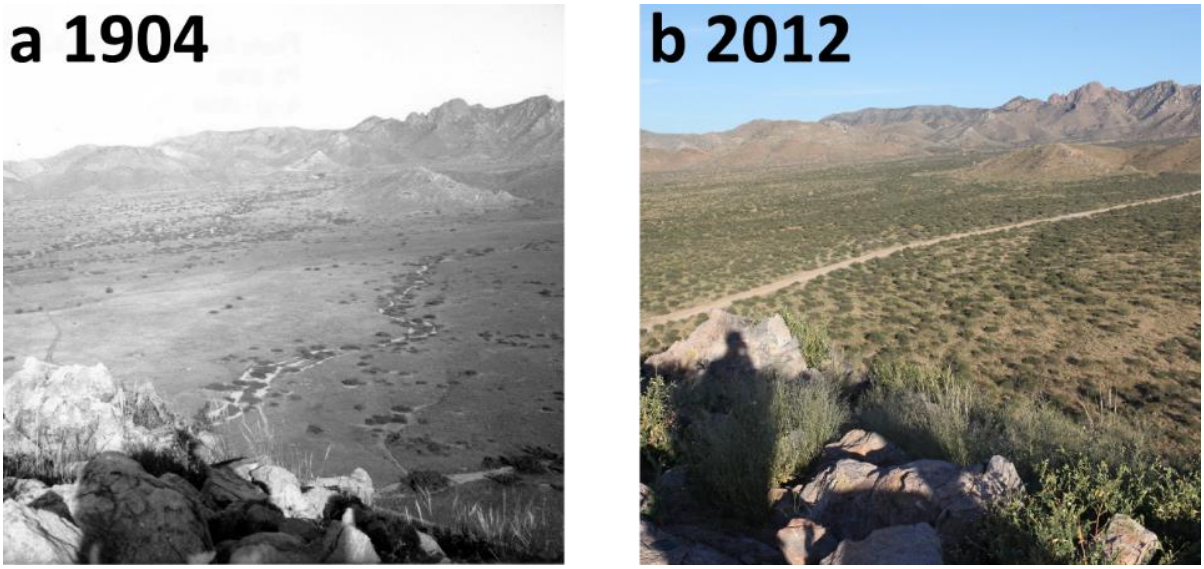

Figure 4.2 Scattered shrubs replace grasses as dominant vegetation between 1904 (a) and 2012 (b) in the Santa Rita Experimental Range, Arizona, US (31˚54' N, 110˚53' W). Facing north-northeast in pastures 6B and 6D (~8 km north of sampling sites). Elevation is 1,228 m asl (public domain images available from https://cals.arizona.edu/srer/photos.html).

4.3.4 Soil characteristics

Soil pH was measured in a 1:5 soil:0.01 M $CaCl_2$ suspension after mixing at 20 rpm for 30 minutes using a Stuart SB3 rotator (Bennett *et al.*, 2003). Particle-size determination for the fine earth fraction (%) was measured using a Malvern Mastersizer 3000 laser particle size analyser with a hydro EV pump accessory (Malvern Panalytical, Worcestershire, UK), for which isopropanol was used as the dispersant (Goossens *et al.*, 2014). Water holding capacity (WHC; %) was estimated using the percentage of sand and clay content of each sample (Saxton and Rawls, 2006), which had been defined according to the USDA-SCS (1982) classification scheme. Soil dry matter content (DMC; %) was determined for all samples using the gravimetric method described by Rowell (1994). Lastly, as it was not possible to extract intact soil cores, an average bulk density value of 1.33 g cm^{-3} was assumed for all samples using previously published data for the SRER (Wheeler *et al.*, 2007).

4.3.5 Elemental analysis

Concentrations of soil organic carbon (SOC) and total N were determined for all samples on an elemental analyser (CHNS-O EA 1108 elemental analyzer; Carlo Erba, Milan, IT) in accordance with methods outlined in section 3.3.4. The detection limits were 100 µg g^{-1} for both elements measured and the coefficient of variation for C and N according to six replicates of an organic analytical standard (NC Soil Standard 338 40025, cert. 133317, C = 2.29 %, N = 0.21 %; Elemental Microanalysis Ltd., UK) were ±1.69 % and ±1.36 %, respectively.

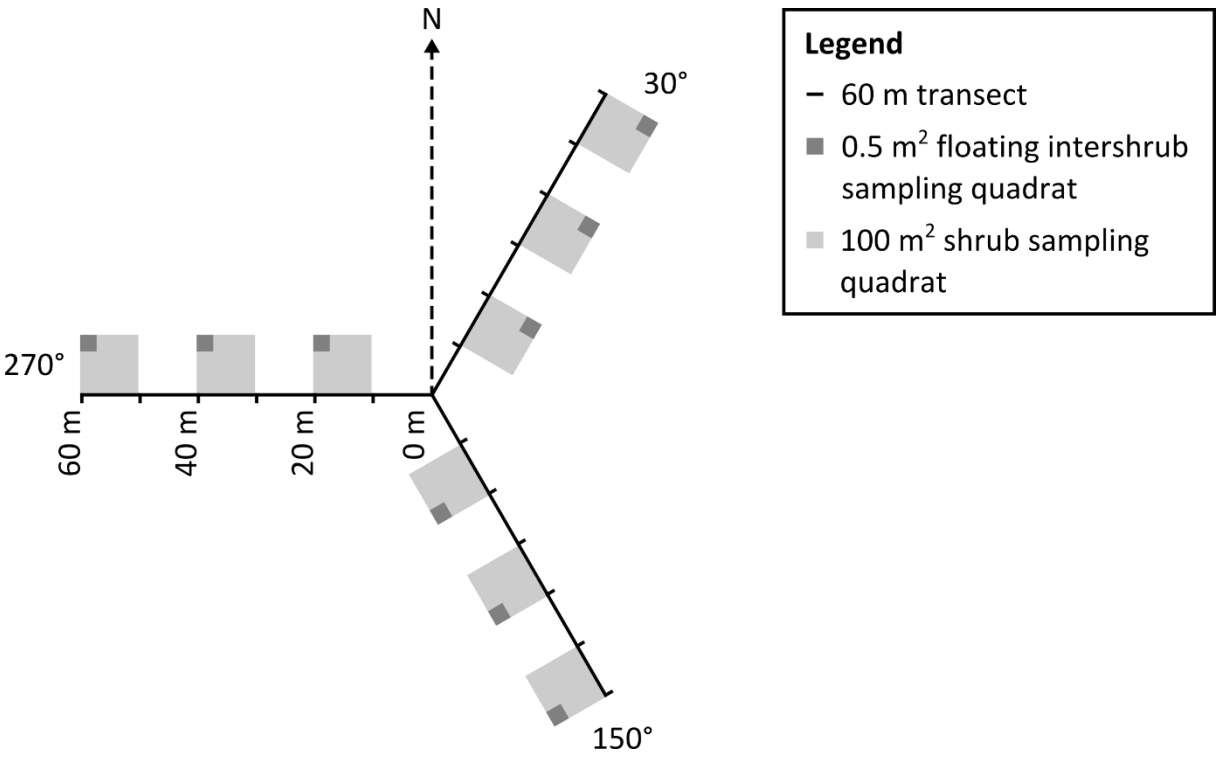

Figure 4.3 Plan view of sampling design in which grass and shrub biomass, as well as intershrub and shrub soil samples, were obtained from 0.5 m^2 and 100 m^2 quadrats, respectively (not to scale). The 0.5 m^2 quadrats were not fixed but were instead centred within the largest intershrub area of each 100 m^2 quadrat.

4.3.6 Soil nutrient analyses

Concentrations of exchangeable NH_4^+-N and NO_3^--N in soil were extracted with 2M KCl (1:10 w/v soil:extractant) and determined colorimetrically using methods outlined in section 3.3.5, for which the coefficient of variation (six replicate standards) for NH_4^+-N and NO_3^--N was ±0.75 % and ±5.19 %, respectively, according to mid-range standards (calibration range: 0-0.5 mg L^{-1}). The respective detection limits (three times the standard deviation of six replicate method blanks) for NH_4^+-N and NO_3^--N were 0.04 µg g^{-1} and 0.08 µg g^{-1} for dry sediment. Bioavailable P (sum of loosely sorbed, and iron- and aluminium-bound P) was sequentially extracted from 0.2 g of soil using a method adapted from Hedley *et al.* (1982) and Mumford (2003) as described in Michaelides *et al.* (2012). The concentrations of the resulting extracts were determined on a Gallery™ Plus automated photometric analyzer (ThermoFisher Scientific, San Jose, CA). The limit of detection (three times the standard deviation of six replicate method blanks) for both loosely sorbed, and iron- and aluminium-bound P was 1.1 µg g^{-1}. The coefficient of variation (six replicate standards) for the same fractions were ±0.35 % and ±0.41 %, respectively. All samples were blank corrected where blank concentrations exceeded the detection limits.

4.3.7 Microbial biomass estimation

PLFAs were extracted from 2 g aliquots of each sample using a modified Bligh and Dyer (1959) method as described by Frostegård *et al.* (1991). Subsequent fractionation and derivatisation was performed according to methods by Dickson *et al.* (2009) and Christie (1993), respectively. The methyl ester of nonadecane was added at a concentration of 0.1 mg L^{-1} as an internal standard to quantify the fatty acids. Individual compounds were quantified using a gas chromatograph (GC) equipped with an Agilent VF-23ms column (60 m x 0.32 mm x 0.15 µm; helium (He) carrier gas, constant 2 mL min^{-1} flow rate), which was operated for 1 min at 50 °C followed by a 10 °C min^{-1} ramp to 100 °C and a 4 °C min^{-1} ramp to a final temperature of 250 °C that was maintained for 15 mins. Compound structures were identified based on comparisons with the retention times and mass spectra for authentic laboratory standards. The mass spectra of individual components were obtained using a Thermo Scientific 1300 Series GC (column, carrier gas, and operation as above) coupled, using a heated transfer line (260 °C), to a Thermo Scientific ISQ LT quadrupole mass spectrometer scanning in the range of *m/z* 50-650 with a dwell time of 0.5 s (current was maintained at 50 µA with an ion source temperature of 240 °C and an electron voltage of 70 eV). Fatty acids which were <14 C and >20 C, or which accounted for <0.5 % of the total peak area, were excluded from the analysis. Bacterial and fungal biomass were defined according to Bååth and Anderson (2003).

4.3.8 Acetylene reduction assay

Due to time and cost constraints, rates of asymbiotic BNF (nitrogenase activity) for intershrub and shrub soils were assessed for three sites (S-4, S-44, and S-56) using the acetylene (C_2H_2) reduction assay technique described in section 3.3.6, where samples were incubated under constant light (0.2 ±0.005 W m^{-2}) at 26 ±0.1 ˚C (mean of maximum and minimum summer temperatures). Daily standards of 100 ppm ethylene (C_2H_4; BOC, Guildford, UK) gave precisions of <5 %. The precision for 100 ppm standards that had been stored in 3.7 mL Exetainers (Labco, Lampeter, UK) for a period of one month also gave precisions of <5 %. Controls for natural C_2H_4 production in the absence of C_2H_2 and C_2H_4 consumption were consistently below the limits of detection. The method was validated using nodulated white clover (*Trifolium repens* L.) root systems, which contain known N-fixing *Rhizobium* spp. (Francis and Alexander, 1972), prior to use in this study (see Appendix B).

4.3.9 Statistical analysis

Statistical analysis of the data was performed using R version 3.5.0. The Kruskal-Wallis *H* test (KW) was used to determine if significant differences occurred for data within the same cover type along the encroachment gradient. When significance was indicated, Dunn's test of multiple comparisons (DT) was applied. The Mann-Whitney *U* test (MW) was used to make within-site comparisons for data among cover types. Relationships between explanatory and response variables were explored using

simple linear regression analysis, for which residuals were inspected for evidence of non-normality using the Shapiro-Wilk test. For this study, the alpha level was set to 0.05. All errors reported in the text are one median absolute deviation about the median (Crawley, 2005).

4.4 Results

4.4.1 Plant biomass

Vegetation biomass and cover were strongly influenced by the degree of shrub encroachment at each site. Overall, total vegetation (grass and shrub) cover decreased from 100 % to 56 % among end-member sites, as reductions in grass cover exceeded gains in shrub cover (Figure 4.1b). The changes in grass and shrub cover were also positively related with grass (R^2 = 0.87; Figure 4.4c) and shrub (R^2 = 0.77; Figure 4.4d) biomass, respectively. Thus, grass biomass declined from 0.62 ±0.19 kg m^{-2} at S-4 to 0 kg m^{-2} at S-56 (Figure 4.4a; DT, $p < 0.001$), whereas shrub biomass increased from 0.02 ±0.02 kg m^{-2} to 4.45 ±4.19 kg m^{-2} among the same sites (DT, $p < 0.001$, Figure 4.4b). Lastly, shrub biomass was greater than grass biomass at all sites except S-4, where grass biomass surpassed shrub biomass ($p < 0.05$).

4.4.2 Soil characteristics

Of the soil properties presented in Table 4.1, only intershrub and shrub soil pH exhibited clear trends with increasing encroachment. Specifically, intershrub soil pH decreased linearly from 6.3 ±0.4 to 4.7 ±0.4 among end-member sites, whereas shrub soil pH increased from 4.9 ±0.1 to 6.0 ±0.2 along the same gradient. Significant differences in the remaining soil properties of Table 4.1 did not follow clear trends and occurred only in intershrub soils. The sand content of intershrub soil at S-28 was lower in comparison with all other intershrub soils (DT, $p < 0.05$). Further, the silt and clay contents of intershrub soil at S-28 were greater, relative to other sites, excluding sites S-55 and S-56, respectively (DT, $p < 0.05$). Lastly, the coarse fraction of intershrub soil at S-55 was greater than that of other intershrub soils along the encroachment gradient (DT, $p < 0.05$).

Soil clay content represented the only consistent physical difference among cover types, for which clay content was higher in soils from intershrub areas relative to those from beneath shrub canopies (MW, $p < 0.05$ for sites S-4 and S-56; $p < 0.01$ for sites S-28, S-44, S-55; Table 4.1). Changes in other physical soil properties did not exhibit a relationship with increasing encroachment. At S-28, the silt content (MW, $p < 0.001$) and DMC (MW, $p < 0.01$) of intershrub soil were greater than those of the shrub soil; however, the sand content of intershrub soil was lower in comparison with shrub soil (MW, $p < 0.001$). Intershrub soil at S-44 exhibited a higher DMC (MW, $p < 0.05$) and sand content (MW, $p < 0.05$) than shrub soil for the same site; though the silt content was lower than that of the shrub soil (MW,

$p < 0.05$). Finally, the percentage of coarse fragments differed between intershrub and shrub soils at S-4 (MW, $p < 0.001$).

4.4.3 Soil carbon and nitrogen

Concentrations of SOC were greater in soils under shrub canopies than soils from intershrub areas at all sites along the encroachment gradient (Figure 4.5a). Further, concentrations of SOC declined from 787 ±398 µg g-1 to 787 ±398 µg g^{-1} between end member sites. In contrast, concentrations of SOC in soils beneath shrub canopies, which ranged from 787 ±398 µg g^{-1} and 1,527 ±171 µg g^{-1}, did not differ among the five sites comprising the degradation gradient (KW, $p > 0.05$).

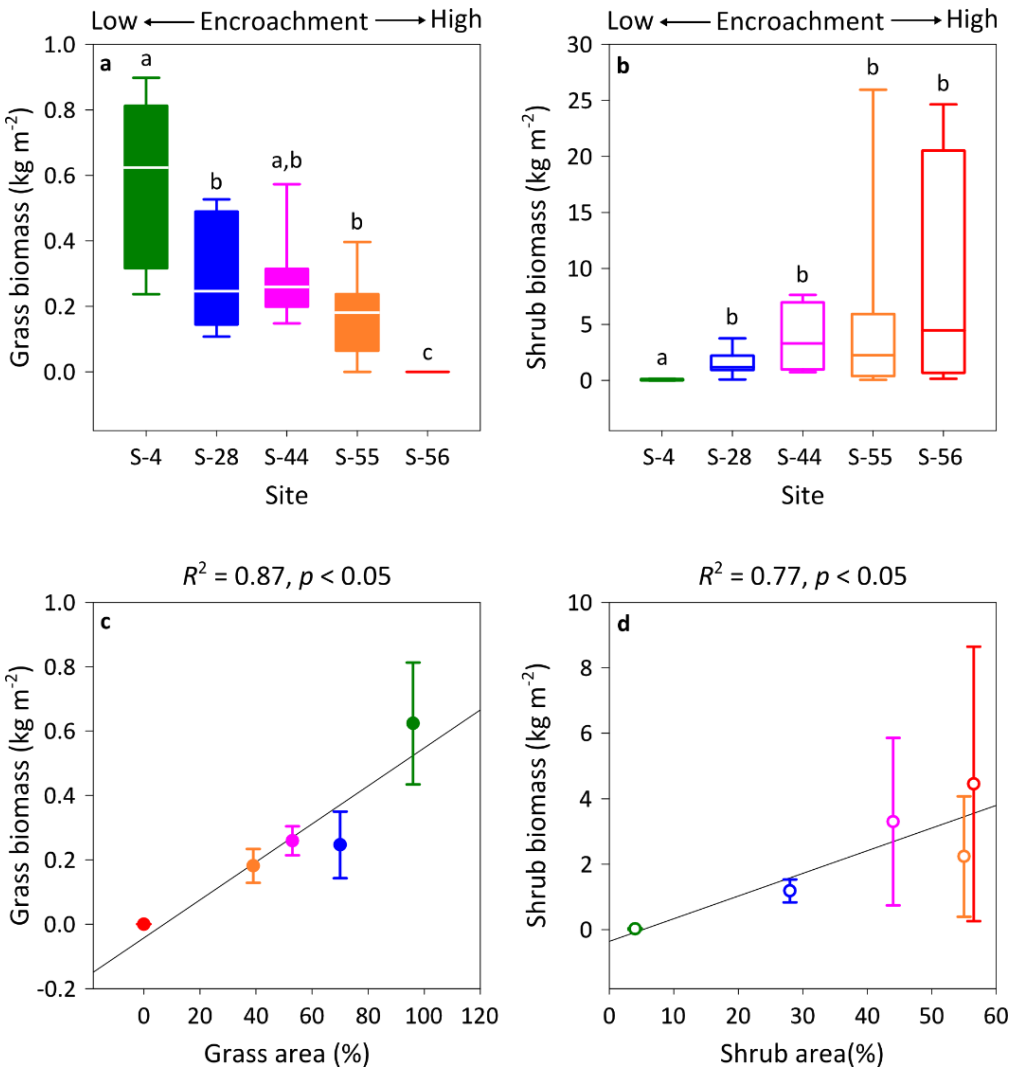

Figure 4.4 Estimated above-ground multispecies grass (a) and *Prosopis velutina* Woot. (b) biomass by dry weight across a shrub encroachment gradient and according to estimated grass (c) and shrub (d) cover in the Santa Rita Experimental Range, AZ, US (n = 9). Boxes denoted with different letters indicate significant differences within biomass classes at $p < 0.05$ (Dunn's test). Error bars represent median absolute deviation about the median.

The total N in shrub soils was more than two times greater than that in intershrub soils (MW, $p < 0.05$) at each site along the degradation gradient (Figure 4.5b). in addition, the total N content of intershrub soils declined proportionally with reductions in grass biomass from 710 ±59 μg g^{-1} at S-4 to 344 ±43 μg g^{-1} at S-56. Specifically, the total N content in intershrub soil at S-56 was lower in comparison with all other intershrub soils (DT, $p < 0.05$), and concentrations of total N within intershrub soils from sites S-44 and S-55 were lower than the concentrations observed at S-4 (DT, $p < 0.05$). Like concentrations of SOC, the total N content of soils under shrub canopies, which was in the range of 787 ±398 μg g^{-1} to 1,527 ±171 μg g^{-1}, did not differ among the sites sampled (KW, $p > 0.05$).

Table 4.1 Median textural characteristics, dry matter content (DMC) and pH (1:5 soil:0.01 M $CaCl_2$) for the surface soil layer (top 10 cm) beneath intershrub (IS) and shrub (S) canopy cover types along a gradient of shrub encroachment in a semi-arid grassland of the Santa Rita Experimental Range, AZ, US. Values in parentheses represent one median absolute deviation about the median.

	Site									
	S-4		S-28		S-44		S-55		S-56	
	IS	S	IS	S	IS	S	IS	S	IS	S
% coarse[a,b,g]	11.4	11.0	18.0	17.6	18.0	18.4	24.7	24.3	16.5	19.3
	(2.7)	(7.1)	(4.8)	(2.9)	(4.0)	(4.8)	(2.3)	(4.6)	(5.0)	(7.6)
% clay[c,d,g]	1.6	0.8	2.2	0.2	1.5	0.1	1.5	0.2	1.7	0.3
	(0.3)	(0.6)	(0.3)	(0.1)	(0.2)	(0.0)	(0.2)	(0.1)	(0.3)	(0.3)
% silt[c,e,g]	41.9	49.5	53.5	36.2	47.9	53.5	35.7	40.3	39.2	35.4
	(3.6)	(15.4)	(3.5)	(3.3)	(8.9)	(3.5)	(5.1)	(5.6)	(6.0)	(6.1)
% sand[c,f,g]	56.9	49.7	44.4	63.7	44.9	44.4	64.2	59.5	57.6	64.3
	(3.1)	(16.0)	(4.2)	(3.3)	(9.5)	(4.2)	(5.0)	(5.8)	(7.4)	(6.3)
% DMC[c,g]	99.1	99.0	99.1	98.9	99.3	98.8	98.9	98.5	98.7	98.8
	(0.1)	(0.1)	(0.0)	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.2)	(0.3)
pH[c,h]	6.3	4.9	5.3	5.7	5.2	5.5	5.1	6.0	4.7	6.0
	(0.4)	(0.1)	(0.1)	(0.5)	(0.1)	(0.3)	(0.1)	(0.0)	(0.5)	(0.2)

[a]total soil (>0 μm)
[b]>2,000 μm
[c]fine earth fraction only (≤2,000 μm)
[d]<2 μm
[e]2-50 μm
[f]>50-2,000 μm
[g]n = 9
[h]n = 3

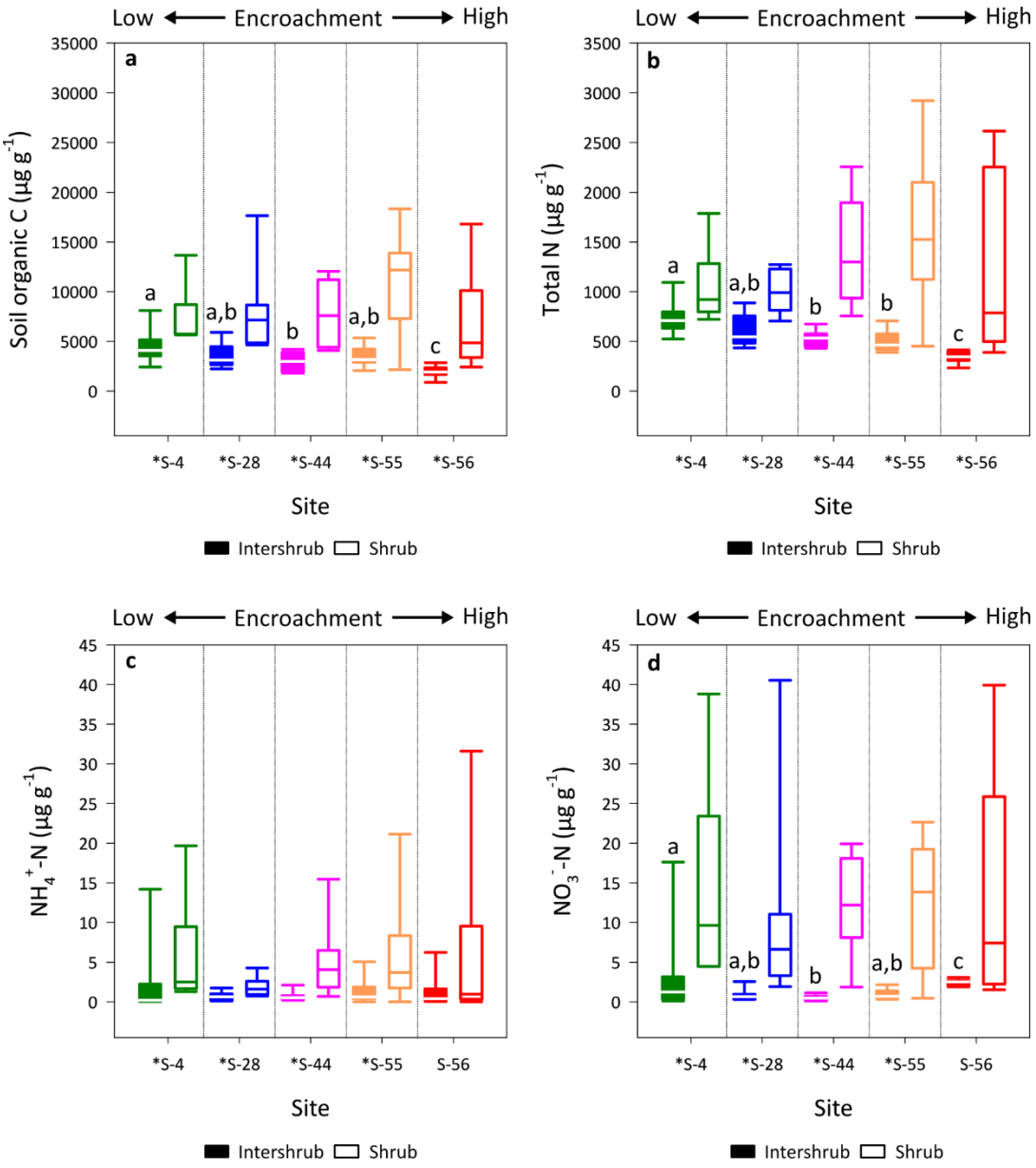

Figure 4.5 Soil organic C (a), total N (b), NH_4^+-N (c) and NO_3^--N (d) concentrations for surface soil (top 10 cm) by dry weight under intershrub and shrub cover types along a shrub encroachment gradient in the Santa Rita Experimental Range, AZ, US (n = 9). Boxes denoted with different letters indicate significant differences by cover type at $p < 0.05$ (Dunn's test). Intershrub and shrub soils at sites denoted with an asterisk differ significantly from each other at $p < 0.05$ (Mann-Whitney *U* test).

Concentrations of NH_4^+-N did not change with increasing encroachment in either the intershrub (KW, $p > 0.05$) or shrub (KW, $p > 0.05$) soils, where the average of median concentrations in intershrub and shrub soils were 1 µg g^{-1} and 3 µg g^{-1}, respectively (Figure 4.5c). However, NH_4^+-N concentrations were approximately an order of magnitude higher under shrub canopies compared to intershrub areas for

all sites (MW, $p < 0.05$) except S-56 (MW, $p > 0.05$). Further, shrub soil NH_4^+-N concentrations exhibited greater variability than those of the intershrub soils.

Like NH_4^+-N, soil NO_3^--N concentrations were greater in soils beneath shrub canopies than in intershrub areas for all sites (MW, $p < 0.05$) except S-56 (MW, $p > 0.05$; Figure 4.5d) and the variability in NO_3^--N in shrub soils was greater than that of their intershrub counterparts. However, unlike NH_4^+-N, intershrub NO_3^--N concentrations varied along the encroachment gradient (KW, $p < 0.001$), where NO_3^--N decreased from 1.2 ±0.8 µg g^{-1} at S-4 to 0.5 ±0.2 µg g^{-1} at S-44, and subsequently rose to 2.5 ±0.2 µg g^{-1} S-56 (Figure 4.5d). No such trend was identified in the NO_3^--N concentrations of shrub soils, which instead remained constant along the encroachment gradient (KW, $p > 0.05$).

To identify site-level changes in the surface N pools, total area-weighted concentrations of total N, NH_4^+-N and NO_3^--N were calculated for each site according to percentage intershrub and shrub cover (Figure 4.1b). Overall, site-level total N concentrations (Figure 4.6a) did not change with increasing shrub encroachment (KW, $p > 0.05$). However, the proportion of total N contained within the inorganic fraction increased in relation to the degree of shrub encroachment. Such increases were largely seen in the form of NO_3^--N (Figure 4.6c), which increased from 0.2 ±0.1 g m^{-2} to 0.6 ±0.3 g m^{-2} between sites S-4 and S-56 (DT, $p < 0.05$). Concentrations of NH_4^+-N experienced an increase from 0.03 ±0.03 g m^{-2} at S-4 to 0.2 ±0.1 g m^{-2} at S-55 (Figure 4.6b), but ultimately did not differ among the end-member sites (DT, $p > 0.05$).

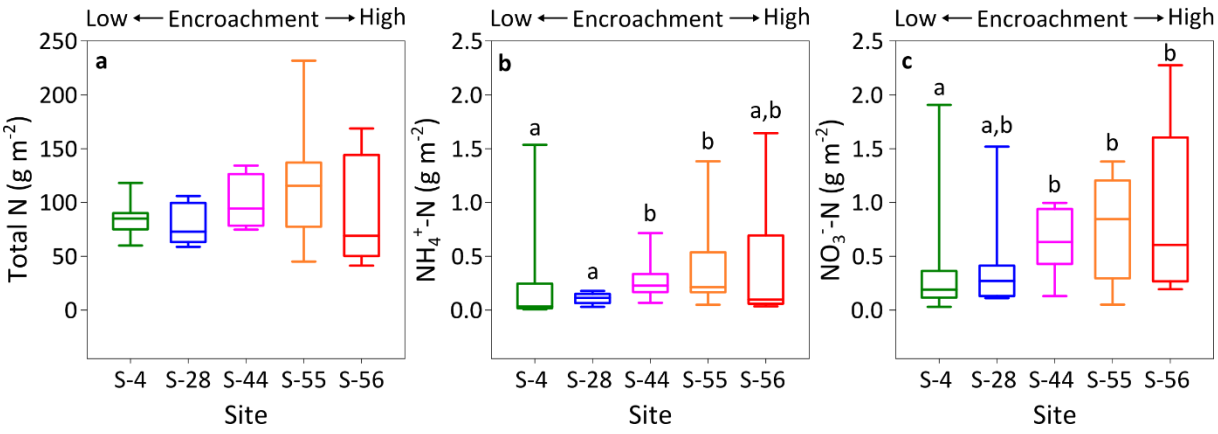

Figure 4.6 Site-level total N (a), NH_4^+-N (b) and NO_3^--N (c) concentrations (dry matter) weighted by relative area of intershrub to shrub cover for surface soil (top 10 cm) along a gradient of shrub encroachment in the Santa Rita Experimental Range, AZ, US (n = 9). Boxes denoted with alternative letters differ significantly from one another at $p < 0.05$ (Dunn's test).

4.4.4 Nutrient ratios

Ratios of SOC:total N (C:N) did not differ across the encroachment gradient for either cover type (KW, $p > 0.05$). The average C:N ratios for intershrub and shrub soils were 7.0 and 7.7, respectively (Figure 4.7a), and these did not differ among cover types at any of the five sites (MW, $p > 0.05$). Similarly, inorganic N:bioavailable P (N:P) ratios (Figure 4.7b) for intershrub ($p > 0.05$) and shrub (KW, $p > 0.05$) soils did not differ significantly among sites, where the respective mean ratios for intershrub and shrub soils were 0.1 and 1.2. However, shrub soil N:P ratios exhibited a high degree of variability and were greater than those of intershrub soils for all sites (MW, $p < 0.05$) except S-56 (MW, $p > 0.05$).

4.4.5 Microbial biomass

No trends were observed in bacterial PLFA biomass across the shrub encroachment gradient (Figure 4.8a). The greatest (3.6 ±0.1 nmol g^{-1}) bacterial PLFA concentrations were found in the intershrub soils at S-44 and the lowest (0.6 ±0.1 nmol g^{-1}) in intershrub soils at S-56. The fungal PLFA biomass of intershrub soils was less variable in comparison with the bacterial PLFA biomass and decreased from a maximum concentration of 0.2 ±0.1 nmol g^{-1} at S-4 across the encroachment gradient, where concentrations were below the limits of detection at both S-28 and S-56 (Figure 4.8b). Similarly, the fungal PLFA content of shrub soils, which was typically greater, but more variable than that of intershrub soils, decreased steadily from 0.3 ±0.1 nmol g^{-1} to 0.05 ±0.05 nmol g^{-1} among end-member sites, where fungal PLFA concentrations were below the limits of detection at S-55. Consequently, the fungal-to-bacterial ratio decreased under both shrub and intershrub soils with increasing shrub encroachment.

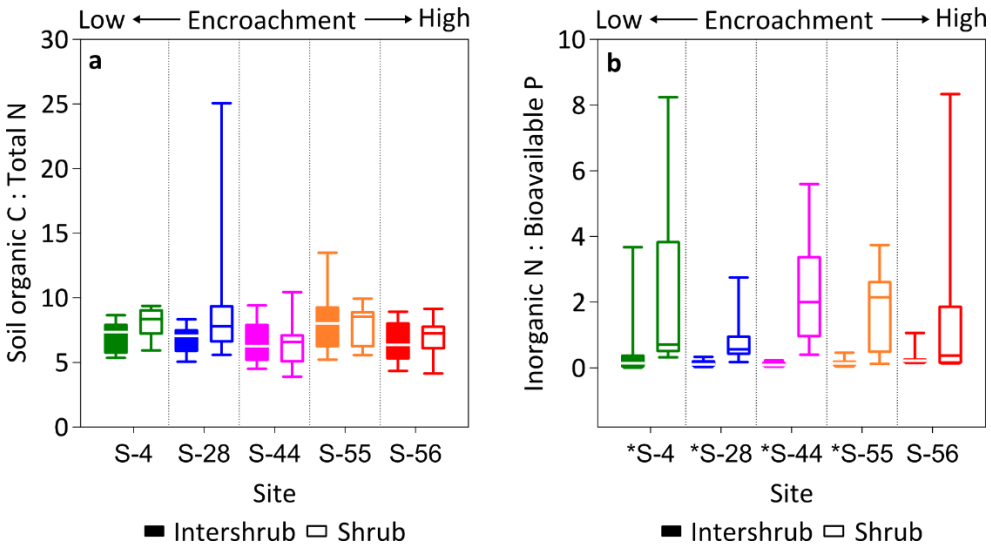

Figure 4.7 Soil organic C : total N (a) and inorganic N : bioavailable P (b) ratios for surface soil (top 10 cm) under intershrub and shrub cover types along a shrub encroachment gradient in the Santa Rita Experimental Range, AZ, US (n = 9). Intershrub and shrub soils at sites denoted with an asterisk differ significantly from each other at $p < 0.05$ (Mann-Whitney *U* test).

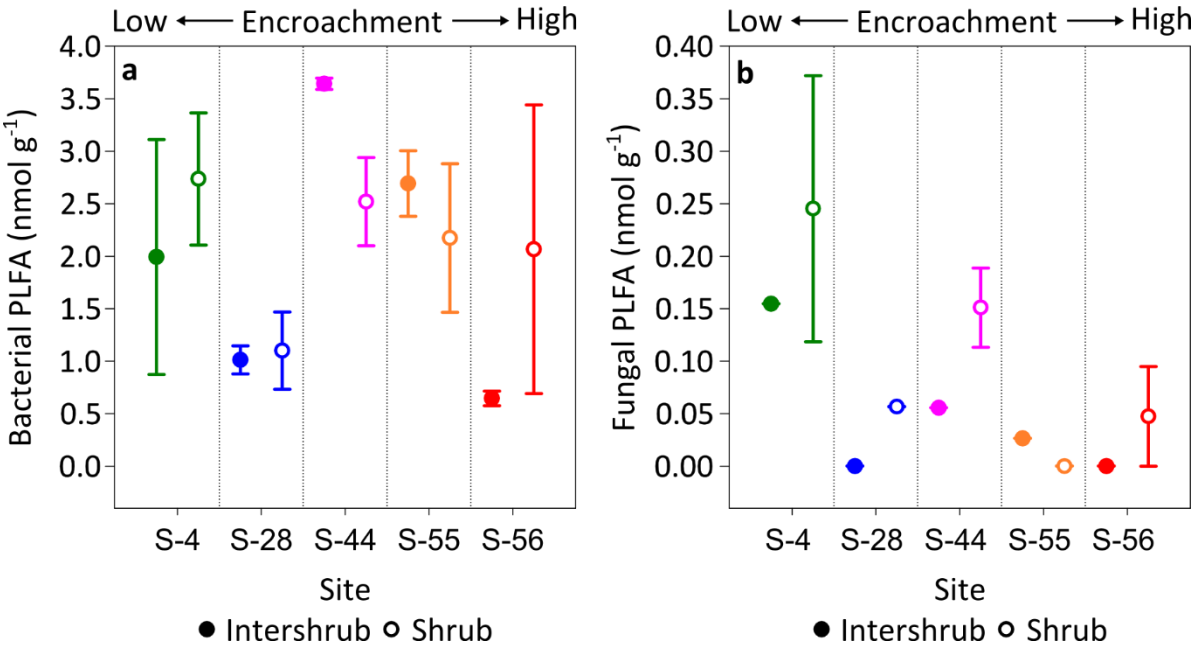

Figure 4.8 Median bacterial (a) and fungal (b) phospholipid fatty acid (PLFA) concentrations for surface soil (top 10 cm) along a gradient of shrub encroachment in the Santa Rita Experimental Range, AZ, US (n = 3). Error bars represent median absolute deviation about the median. All results are reported on a dry matter basis.

4.4.6 Acetylene reduction assay

Rates of C_2H_2 reduction were only above the limits of detection in intershrub soils at sites S-4 and S-44, where rates of C_2H_4 production were linear over the 24-hour incubation period (R^2 = 0.97 for S-4, and R^2 = 0.84 for S-44; slopes of regression lines were significantly different from zero for both S-4 ($p < 0.001$) and S-44 ($p < 0.01$); data not shown here). When converted to annual rates of N fixation per unit area (Figure 4.9a), inputs of N by asymbiotic BNF were similar between sites S-4 (2.4 g N m^{-2} a^{-1}) and S-44 (2.5 g N m^{-2} a^{-1}). However, when adjusted for percentage cover (Figure 4.9b), rates of asymbiotic BNF declined in relation to reductions in intershrub area from 2.3 g N m^{-2} a^{-1} at S-4 to 1.4 g N m^{-2} a^{-1} at S-44.

4.5 Discussion

Our study revealed the distribution and speciation of soil N, as well as the extent of nitrogenase activity, are strongly influenced by the degree of shrub encroachment in a semi-arid grassland of the SRER. Yet, overall, the soil N pool incurred no net change with the transition from a grass- to shrub-dominated state. Below we discuss specifically how the soil N pool responds to increasing shrub cover, and how changes in the soil microbial biomass and nutrient ratios associated with shrub encroachment influence N inputs by asymbiotic BNF in a semi-arid grassland.

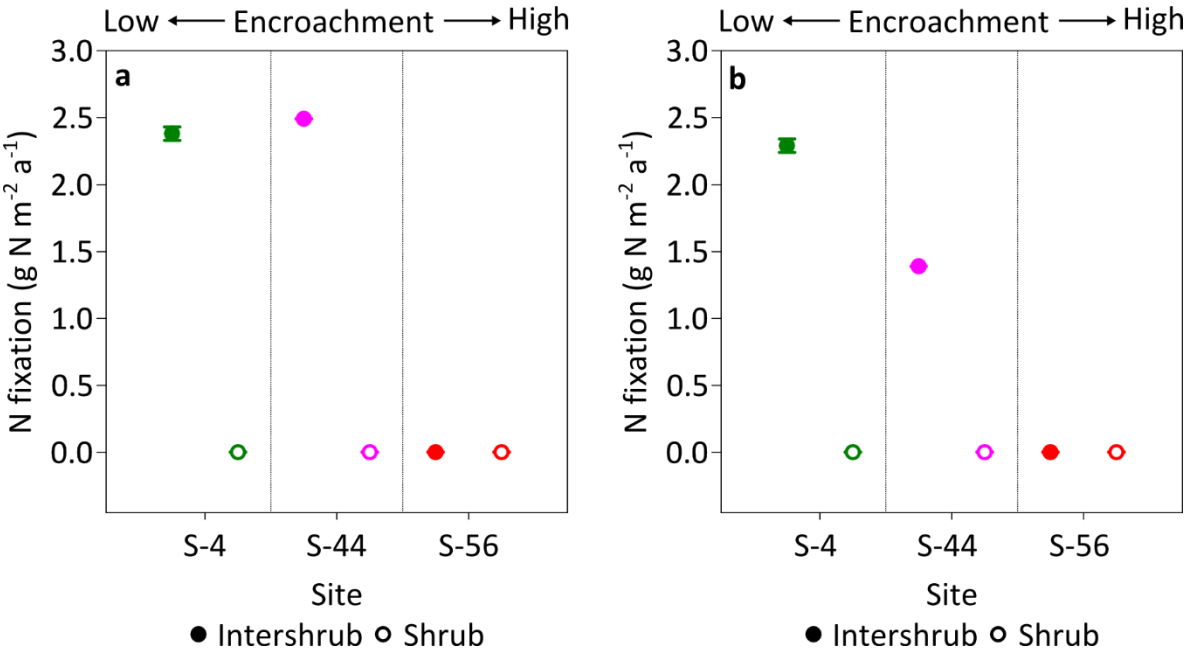

Figure 4.9 Median rates of asymbiotic nitrogen (N) fixation for intershrub and shrub surface (top 10 cm) soil (a) and intershrub and shrub surface soil weighted by area cover (b) along a gradient of shrub encroachment in the Santa Rita Experimental Range, AZ, US (n = 3). Error bars represent median absolute deviation about the median. N fixation rates were determined using the acetylene reduction assay method in which ethylene was converted to N_2 using a 3:1 ratio (Telling *et al.*, 2011). Results are reported on a dry matter basis.

4.5.1 Distribution and speciation of soil nitrogen

Like other studies for the southwestern US (e.g., Michaelides *et al.*, 2012; Schlesinger *et al.*, 1996; Turnbull *et al.*, 2010; Wheeler *et al.*, 2007), we determined that concentrations of total N are greater in surface soils under shrubs relative to intershrub areas for all sites along the encroachment gradient (Figure 4.5b-d). Further, whilst concentrations of total N in shrub soils are not statistically separable, we found the total N content of intershrub soils declined among end-member sites coincident with reductions in herbaceous biomass (Figure 4.4a) and cover (Figure 4.1b). As site-level concentrations of total N remain constant with increasing shrub cover (Figure 4.6a), the accumulation of N beneath shrub canopies can be considered a direct consequence of N losses from intershrub soils. These findings contrast with studies which identified net increases (e.g., Boutton and Liao, 2010) and declines (e.g., Jackson *et al.*, 2002) in the total N pool with shrub encroachment but support the widely held perspective that transitions from continuous grass- to shrub-dominated states increase the spatial heterogeneity of soil resources (D'Odorico *et al.*, 2013; Peters *et al.*, 2006; Schlesinger *et al.*, 1990).

Organic N (total N minus inorganic N) comprises >98 % of total N in all intershrub and shrub soils (Figure 4.5b-d). Thus, the elevated concentrations of total N in shrub soils, which are ≥1.5 times those of intershrub soils, represent larger stores of mineralisable N. Rates of N mineralisation are inversely related to soil C:N ratios (Finzi *et al.*, 1998), which in turn have been shown to decrease under mesquite canopies in response to inputs of higher-quality litter (Geesing *et al.*, 2000; Throop and Archer, 2007). However, like Wheeler *et al.* (2007), we found that soil C:N ratios, which are lower than those previously reported for the area (Wheeler *et al.*, 2007), are not affected by shrub encroachment processes in the SRER (Figure 4.7a). Whilst this could be due in part to depressed subcanopy soil-litter mixing (Throop and Archer, 2008), it is possible that the lower clay content in soils under shrubs relative to those from intershrub areas may limit the accumulation N (Table 4.1). This is because the accumulation (and flux) of N has been shown to share a positive relationship with the soil clay content due to the tendency of organic and inorganic (i.e., NH_4^+-N) forms of N to adsorb onto clay minerals (Michaelides *et al.*, 2012; Virginia and Jarrell, 1983). Nevertheless, shrub soils contain greater concentrations of NH_4^+-N and NO_3^--N than intershrub soils at all sites except S-56 (Figure 4.5c,d), which suggests the microbial community beneath shrub canopies can mineralise organic forms of N more readily. This is supported by a study for a semi-arid grassland in central Spain, which showed the availability of inorganic N is largely influenced by the abundance and functional diversity of the microbial community (Delgado-Baquerizo *et al.*, 2015).

In this study, bacterial PLFA concentrations did not follow a clear trend with increasing shrub encroachment (Figure 4.8a), whereas the fungal PLFA content of both intershrub and shrub soils decreased linearly among end-member sites (Figure 4.8b). These results contrast with previous studies, which found the microbial biomass to be greater in soils beneath shrub canopies than adjacent grasses due to reduced environmental stress and greater stores of SOM (Ewing *et al.*, 2007; Gallardo and Schlesinger, 1992; Li *et al.*, 2017). As ratios between the fungal and bacterial constituents of the microbial community share a negative relationship with soil pH (Bååth and Anderson, 2003), a decline in the fungal PLFA content of shrub soils can be attributed to changes in shrub soil pH, which increased with gains in shrub cover (Table 4.1). However, the same cannot be ascribed to changes in the fungal PLFA content of intershrub soils, as declines among end-member sites occurred with decreases in soil pH. Instead, if concentrations of SOC are considered (Figure 4.5a), reductions in intershrub fungal PLFA concentrations with increasing encroachment may be indicative of C limitation for growth, which would be expected to reduce the microbial biomass and release inorganic N (Cookson *et al.*, 2006). Accordingly, in addition to an absence of fungal PLFAs, intershrub soil at S-56 exhibits the lowest bacterial PLFA content in comparison with all other soils along the encroachment gradient, as well as the highest concentration NO_3^--N within the intershrub soils (Figure 4.5d). As fungi have been shown to preferentially utilise grassland C (Creamer *et al.*, 2016), declines in the shrub soil fungal PLFA content

with increasing encroachment may also be a response to the progressive depletion of grass-derived SOC.

It must be noted that we recommend caution when interpreting the vegetation cover and biomass estimates presented here, as well as data weighted by the percentages of herbaceous and woody cover. The classification accuracy of cover estimates, which are subject to image spatial resolution, as well as land-cover spatial and spectral heterogeneity (Hu *et al.*, 2013), were not formally assessed. Likewise, woody and herbaceous biomass estimates were derived from allometric relationships that had not been ground-truthed and which could potentially vary by soil type, surface morphology and disturbance history (McClaran *et al.*, 2013; Nafus *et al.*, 2009). Had time and funding not been limiting, we recognise that improved vegetation cover and biomass estimates could have been attained from the application of a structure-from-motion photogrammetry technique developed by Cunliffe *et al.* (2016a). Using this technique, Cunliffe *et al*. (2016a) demonstrated that three-dimensional models produced from photographs captured by unpiloted aerial systems can be used to describe the landscape vegetation structure of semi-arid drylands in the southwestern US at ultra-fine (<1 cm^2) resolutions. Further, these models can be used to estimate above-ground woody (e.g., *Juniperus monosperma* (Engelm.) Sarg. and *Larrea tridentata* (DC.) Coville) and herbaceous (e.g., *B. eripoda* (Torr.) Torr. and *B. gracilis* (Willd. ex Kunth) Lag. ex Griffiths) biomass (*ibid*.). Nevertheless, as relative changes in estimated percentage grass and shrub cover can explain concurrent changes in estimated grass and shrub biomass (Figure 4.4c, d), we argue the vegetation cover and biomass estimates presented in this study are still useful for capturing the extent of shrub encroachment at each site.

In addition to concerns regarding vegetation cover and biomass estimates, we recognise that the SOC and total N data contained within this study may have been limited by excluding the coarse fraction (>2,000 µm) of soils from our analyses. This is because, despite not typically being included in soil C and N analyses (e.g., Bernasconi *et al*., 2011; Dümig *et al*., 2011; Throop and Archer, 2008; Turnbull *et al*., 2010; Wheeler *et al*., 2007), studies have shown that coarser grained materials may accumulate significant amounts of these elements with pedogenesis (e.g., Agnelli *et al*., 2001; Corti *et al*., 2002; Cunliffe *et al*., 2016b). Specifically, a study by Cunliffe *et al*. (2016b) showed that the coarse fraction of surface soils from a semi-arid dryland in the southwestern US comprised ~24-38 % of the SOC stock in that area. However, whilst it is possible that we may have underestimated the concentrations of SOC and total N in the SRER soils, it is unlikely that including soil particles >2,000 µm within our analyses would have impacted significantly upon the trends identified here as the coarse fraction only differs among intershrub sites and between intershrub and shrub soils at S-55 (Table 4.1). Incidentally, we found no evidence for a coarsening of intershrub soils relative to soils under shrub canopies with reductions in herbaceous cover (Table 4.1). This contradicts studies (e.g., Abrahams *et al.*, 1995; Michaelides *et al.*, 2012) which have shown that increases in runoff and erosion with declines in

herbaceous cover cause the clay fraction of intershrub soils, which is typically associated with majority of soil N (Michaelides *et al.*, 2012), to be redistributed to soils under shrub canopies by decreasing resistance to overland flow and run-on infiltration.

4.5.2 Inputs of asymbiotically fixed nitrogen

In this study, we explored a gap in the literature concerning the impacts of increasing shrub encroachment (at sites S-4, S-44 and S-56 only) on the rates and distribution of asymbiotic BNF in a semi-arid grassland. We found that asymbiotic BNF was only detectable in intershrub soils at sites S-4 and S-44 (Figure 4.9a), which exhibited significantly lower N:P ratios than adjacent shrub soils (Figure 4.7b). These findings reinforce the theory that increases in inorganic N relative to available P inhibit nitrogenase activity, the enzyme responsible for mediating the adenosine triphosphate reduction of N_2 to ammonia in BNF (Smith, 1992). However, despite intershrub soil N:P ratios remaining constant with increasing encroachment, there was an absence of asymbiotic BNF at S-56. As asymbiotic diazotrophs (N-fixing organisms) are largely contained within the bacterial fraction of the microbial biomass (Poly *et al.*, 2001), the undetectable rates of asymbiotic BNF at S-56 could be attributed to the low bacterial PLFA concentration at this site (Figure 4.8a). This conclusion is supported by increases in asymbiotic BNF rates with gains in the bacterial PLFA content of intershrub soils between sites S-4 and S-44.

When weighted by intershrub area, rates of asymbiotic BNF declined from 2.3 g N m^{-2} a^{-1} at S-4 to 1.4 g N m^{-2} a^{-1} at S-44 (Figure 4.9b). As levels of total N deposition (0.1-0.2 g N m^{-2} a^{-1}) in the southwestern US are generally low (Schwede and Lear, 2014), the decreasing rates of BNF associated with increasing site-level concentrations of inorganic N (Figure 4.6b, c) with shrub encroachment represent the loss of a significant N input pathway to these already N-limited systems, which Belnap (1995) argues may not be readily recovered. However, the rates of asymbiotic BNF reported here, which exceed previous estimates for dryland systems by an order of magnitude (Cleveland *et al.*, 1999), represent an absolute upper limit as soil moisture, temperature and light were maintained during the incubation experiments. Constant incubation parameters were used to achieve linear rates of C_2H_2 reduction over a 24-hour period, in which parameters were chosen to reflect mean summer conditions when microbial activity is greatest due to increased soil water availability (Loik *et al.*, 2004).

A study by Geesing *et al.* (2000) showed the development of the soil N pool beneath mesquite canopies is attributable to inputs of symbiotically fixed N. As site-level concentrations of total N do not differ between end-member sites, we argue the loss of intershrub soil N and asymbiotic BNF with shrub encroachment seen here is balanced by inputs of symbiotically fixed N by mesquite. However, as the redistribution of soil resources with shrub encroachment modifies the development of shrub over intershrub soils, inputs of N by mesquite-*Rhizobium* symbioses are ultimately unavailable to sustain

grass production in intershrub areas. Consequently, transitions from grass- to shrub-dominated semi-arid systems may be viewed as a shift between bistable states from which intershrub areas may not readily recover (D'Odorico *et al.*, 2013).

4.6 Conclusions

Over the past century, the semi-arid grasslands of the southwestern US have been degraded by the encroachment of mesquite, an N-fixing shrub. From this comprehensive study, we found that as declines in the intershrub soil N pool were proportional to increases in soils under shrub canopies, the site-level soil N pool remained constant along a gradient of increasing shrub cover within a semi-arid grassland of the SRER. Yet the inorganic fraction of the site-level soil N pool increased with shrub area and biomass due to the long-term accumulation of NH_4^+-N and NO_3^--N in soils beneath shrub canopies. As soil C:N ratios did not differ among sites or cover type, increases in concentrations of inorganic N in shrub soils were attributed to the capacity of the soil microbial community to mineralise organic forms of N. Finally, as the ratio of inorganic N to bioavailable P has been shown to influence nitrogenase activity, we explored the effects of shrub encroachment on the distribution and rates of asymbiotic BNF in the grassland soil. We found that BNF was inhibited by higher concentrations of inorganic N to available P and, when weighted by intershrub area, provided a lower input of new N to the system with increasing shrub cover. The loss of this ecosystem process, as well as declines in the intershrub soil N pool, were potentially balanced by inputs of symbiotically fixed N by mesquite. Consequently, we conclude the long-term accumulation of N beneath shrub canopies combined with increases in shrub canopy area have the potential to significantly change the dynamics of soil N cycling in dryland systems.

4.7 Acknowledgements

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5 General discussion

5.1 Impacts of environmental change on soil nitrogen

Nitrogen (N) is the most significant limiting nutrient of primary production in drylands over physiological timescales (Gebauer and Ehleringer, 2000). Mediated almost exclusively by microbiological processes (Robertson and Groffman, 2015), N cycling is sensitive to the climate- and land use-driven environmental changes which have been occurring in arid regions over the past ca. 150 years (Delgado-Baquerizo *et al.*, 2013; Maestre *et al.*, 2012a). In water-limited grasslands, environmental change in the form of woody plant encroachment is widely recognised as a significant form of land degradation (Eldridge *et al.*, 2011) that alters the spatial distribution and fluxes of soil N (Browning *et al.*, 2008). In contrast, declines in ice-cover associated with climate warming in high latitude and altitude arid regions are exposing primary mineral substrates previously overridden by ice to successional processes and the subsequent accumulation of N (Bradley *et al.*, 2014). When considering drylands are projected to occupy ~50 % of Earth's land surface by 2100 (Huang *et al.*, 2016), changes within soil N pool has implications for the global carbon (C) cycle, as fluxes of C are largely modulated by processes associated with primary productivity (Butterbach-Bahl *et al.*, 2011). Thus, the aim of this research project was to improve current understanding of how the soil N pool responds to environmental changes between trajectories of succession and degradation in arid ecosystems, which were delineated according to the methods outlined in section 1.1.4.

The results from the meta-analysis presented in Chapter 2 of this thesis demonstrate that the total N contents of surface soils from emerging ice-free arid ecosystems and shrub-encroached drylands are influenced significantly by surface cover (Figure 2.4a). Specifically, it was established that the lowest concentrations of total N typically occur in bare soils, whilst the highest are found in soils under woody vegetation. Further, the total N content of soils under both non-vascular and herbaceous cover were determined not to differ between emerging ice-free arid ecosystems and shrub-encroached drylands. Yet despite these similarities, a comparison of the results in chapters 3 and 4, which were not included within the meta-analysis due to being unpublished at the time of literature searches, reveal that the consequences of surface-cover changes on the spatial distribution of N differ between trajectories of succession and degradation in arid ecosystems.

The primary study presented in Chapter 3 of this thesis shows that the total N pool increases with time on soil development in the forefield of a high latitude glacier (Midtre Lovénbreen, Svalbard, Norway) in the polar tundra Köppen-Geiger (K-G) climate class (Figure 3.4b). Increases in total N concentrations between the 0- (84 ±12 μg g^{-1}) and 50-year-old (215 ±48 μg g^{-1}) soils are likely to be attributable to higher aggregate stability (Maestre *et al.*, 2005) resulting from the colonisation of soils exposed for ≥16 years by biological soil crusts (Hodkinson *et al.*, 2003). The accumulation of N subsequently

accelerates with the colonisation of the surface by herbaceous plants in soils 113 (606 ±70 μg g^{-1}) and 2,000 (4,399 ±498 μg g^{-1}) years old, which is to be expected as the roots of vascular plants deliver higher inputs of nutrient-rich rhizodeposits to the soil system (Duc *et al.*, 2009). Thus, if viewed as a linear trajectory of primary succession, the total N content of soil increases with surface cover change in the order bare soil < biological soil crust (i.e., non-vascular) < herbaceous, which suggests that the fertility of glacier forefield ecosystems will increase with time on soil development. Further, if data for the 2,000-year-old soils in Chapter 3 are excluded, as the forefield studies aggregated for meta-analysis rarely contained data for soils older than ca. 150 years, these findings can be seen to validate the median total N concentrations for bare soil (140 ±130 μg g^{-1}), non-vascular (350 ±230 μg g^{-1}) and herbaceous (590 ±475 μg g^{-1}) cover types in the polar tundra K-G climate class presented in Chapter 2.

In contrast to soil development in a glacier forefield, the results presented in Chapter 4 show that surface-cover changes associated with woody plant encroachment did not increase the total N pool in soil from a water-limited grassland ecosystem (Santa Rita Experimental Range, Arizona, US) in the hot arid steppe K-G climate class (Figure 4.6a). Instead, whilst N losses through overland flow and run-on infiltration may have occurred (Michaelides *et al.*, 2012; Wainwright *et al.*, 2000), the total N pool was determined to be progressively redistributed from intershrub areas to soils beneath shrub canopies along a gradient of increasing woody plant encroachment (Figure 4.5b). Specifically, concentrations of total N in intershrub areas decline with reductions in herbaceous cover (Figure 4.4c) from 710 ±56 μg g^{-1} to 344 ±43 μg g^{-1} between end-member sites (i.e., a transition from herbaceous to bare soil cover in intershrub areas), whereas the total N content of soils under expanding shrub canopies remain constant at ~1,165 μg g^{-1}. These findings are corroborated by comparable total N concentrations for soils under bare (470 ±22 μg g^{-1}), herbaceous (700 ±177 μg g^{-1}) and woody (845 ±341 μg g^{-1}) cover types in the hot arid steppe K-G climate class shown in Chapter 2. Despite not affecting concentrations at the ecosystem-level, this redistribution of the total N pool with woody plant encroachment creates a positive feedback that contributes to the continued expansion of shrub functional types and inhibits the recovery of intershrub areas (D'Odorico *et al.*, 2013). Consequently, the resulting shift between bistable states, which may not be readily reversed over human timescales, can be viewed as a form of land degradation as it reduces pastoral productivity (Eldridge *et al.*, 2011).

A parallel between the results in chapters 3 and 4 is that ≥98 % of total N in soil is typically contained within the organic phase (total N minus ammonium-N and nitrate-N). These findings are supported by the meta-analysis in Chapter 2 which determined that >98 % of N in soils from both emerging ice-free arid ecosystems and shrub-encroached drylands is organically bound. This suggests that N is readily immobilised by the soil microbial biomass due to N-limiting conditions (Vitousek and Howarth, 1991). Thus, the capacity for biological N fixation (BNF) may represent a selective advantage in these nutrient-poor arid ecosystems (Vitousek *et al.*, 2002a).

The biological fixation of atmospheric dinitrogen by asymbiotic diazotrophs is an important input pathway of reactive N to dryland ecosystems (Bradley *et al.*, 2014; Evans and Ehleringer, 1993). Yet the factors influencing the extent and distribution of this N input process are poorly resolved. Using the acetylene reduction assay technique, the results of chapters 3 and 4 show that inputs of N by asymbiotic BNF are generally limited to soils which exhibit N:P ratios of <0.3. Consequently, these findings support the theory that BNF rates will downregulate as the availability of N increases relative to P due to the high demand of the latter for adenosine triphosphate (Smith, 1992). However, the influence of N:P ratios on BNF rates may be complicated by additional factors.

Despite an N:P ratio of 0.2 in 29-year-old soil (Figure 3.5b), BNF was only identified in the 0- and 3-year-old soils from the Midtre Lovénbreen glacier forefield (Figure 3.6a-c). As the 29-year-old soil is associated with areas in the glacier forefield colonised by biological soil crusts, it can be argued that rates of asymbiotic BNF are controlled by complex interactions between surface-cover type and the availability of N and P. Indeed, potential BNF rates decrease respectively from 2.3 g N m^{-2} a^{-1} to 0 g N m^{-2} a^{-1} between soils with similar N:P ratios (Figure 4.7b) under herbaceous and bare cover types in a hot arid steppe grassland of the Santa Rita Experimental Range. Whilst N-limiting conditions should promote BNF, declining nitrogenase activity with time on soil development in the Midtre Lovénbreen glacier forefield, as well as soils under shrub canopies in the Santa Rita Experimental Range, can most likely be attributed to gains in the total N pool with surface-cover change which would favour heterotrophic bacteria and fungi capable of mineralising organic N to inorganic forms (Bradley *et al.*, 2014). In contrast, the lack of capacity for BNF in bare intershrub soils from the Santa Rita Experimental Range is a probable consequence of a reduction in the bacterial biomass (Figure 4.8a) due to increased environmental stresses (Creamer *et al.*, 2016; Poly *et al.*, 2001).

The implications concerning the distribution of BNF vary between trajectories of succession and degradation. The primary study in Chapter 3 demonstrates that asymbiotic BNF is an important N input pathway to the Midtre Lovénbreen glacier forefield during the initial stages of soil development. In contrast, the primary study in Chapter 4 suggests that a reduction in the capacity for BNF in intershrub soils with increasing bare soil and woody cover may expedite grassland-to-shrubland transitions as N losses from intershrub areas will not be replenished.

5.2 Main conclusions

The research presented in this thesis addresses a gap in the literature concerning a comparative assessment of the effects of environmental changes on the soil N pool between trajectories of succession and degradation in emerging ice-free arid ecosystems and shrub-encroached drylands, respectively. From this research it was determined that concentrations of total N in soils from both emerging ice-free arid ecosystems and shrub-encroached drylands increase with changes in surface

cover in the order bare soil, non-vascular, herbaceous and woody. However, major differences arise between pathways of degradation and succession. Increasing concentrations of total N with soil age and surface-cover change in a high latitude glacier forefield suggest that emerging ice-free arid ecosystems will become more productive with time on soil development and primary succession (Figure 5.1a). In contrast, the progressive redistribution of the total N pool from intershrub areas to soils beneath shrub canopies was determined not enhance soil fertility overall. Instead, the development of fertility islands beneath shrub canopies contribute to a decline in pastoral productivity as reductions in nutrient availability and microbial activity are insufficient to sustain herbaceous biomass for grazing in intershrub areas (Figure 5.1b). Despite these differences, it was found that >98 % of total N in both emerging ice-free arid ecosystems and shrub-encroached drylands is contained in organic forms, which suggests that N is readily immobilised by soil microbial biomass due conditions of N limitation. Thus, the capacity for BNF should represent a selective advantage in these ecosystems. Using the acetylene reduction assay technique, inputs of N by asymbiotic BNF were determined to be important to the early stages of soil development in a high latitude glacier forefield and maintaining productivity in intershrub areas in a hot arid steppe grassland. Whilst potential rates of asymbiotic BNF were found to be largely controlled by the availability of inorganic N relative to bioavailable P, nitrogenase activity declined in soils from a polar tundra glacier forefield and areas beneath shrub canopies in a hot arid steppe grassland with the accumulation of total N. As the majority of soil N is organically bound, this suggests that the accumulation of organic matter is a significant driver of the soil microbial community in arid ecosystems. Overall, this research provides a greater insight into how environmental changes associated with succession and degradation influence the soil N pool in arid ecosystems.

5.3 Methodological considerations

Of the studies returned by literature searches, only 70 from 434 for emerging ice-free arid ecosystems and 48 from 436 for shrub-encroached drylands were accepted for meta-analysis in Chapter 2 of this thesis. Whilst beneficial for capturing relevant studies associated with alternative text words (Atkinson and Cipriani, 2018), it was understood that combining Boolean operators with broader search terms (see section 2.3.2) would return numerous sources unrelated to either zones of aridity as delineated according to Peel *et al.* (2007; Figure 2.1; e.g., "ice-free" not limited to arid ecosystems), or the elements (e.g., “nutrient*” not limited to C or N) and environmental changes (e.g., “invasion” not limited to woody plant encroachment) of interest. However, as just 18 % and 11 % of studies identified for emerging ice-free arid ecosystems and shrub-encroached drylands, respectively, were accepted, it is feasible that the inclusion criteria used to evaluate the appropriateness of studies for meta-analysis were too restrictive (see section 2.3.2).

Requiring studies to state the cover type of sampling sites was deemed necessary to facilitate comparisons of soil N responses to environmental changes between emerging ice-free arid ecosystems and shrub-encroached drylands. As emerging ice-free ecosystems, such as glacier forefields, are widely recognised for their potential to explore ecological succession linearly over a variety of timescales, it is common for studies in such areas to apply line transect methods which capture and/or consider the effects of surface-cover changes on soil nutrient pools (Bradley *et al.*, 2014). In contrast, there is no single methodological approach commonly applied to assess the effects of woody plant encroachment on soil nutrient pools in water-limited grassland ecosystems. For example, some studies (e.g., Yusuf *et al.*, 2015) have reported mean C and N concentrations by mass per unit for entire plots along gradients of increasing shrub density, where samples were collected randomly irrespective of surface cover. Such studies could not be accepted for meta-analysis in Chapter 2 because their sampling strategy did not consider, or report results by cover type. Thus, it can be argued that more studies may have been accepted if the data aggregated for meta-analysis were presented in this manner (i.e., average nutrient concentrations for ecosystems by dominant cover type). However, even if the information necessary to perform these calculations were available (i.e., soil depth and bulk density, and vegetation patch-size), it is likely that bare soil and non-vascular cover types would not be represented in the study as neither have been shown to dominate ecosystems undergoing shrub encroachment (e.g., Turpin-Jelfs *et al.*, 2019a; Chapter 4 in this thesis).

In addition to concerns regarding the cover type criterion, it is possible that the number of accepted data sources for meta-analysis were limited by the methods used to delineate between arid regions. The world map of K-G climate classification produced by Peel *et al.* (2007; Figure 2.1) exhibits a relatively low resolution of 0.1° and fails to consider the topographical factors which modulate temperature and precipitation in high-altitude regions (Beck *et al.*, 2018). Thus, it is possible relevant studies, particularly in mountainous environments, were rejected unnecessarily. Nevertheless, the map produced by Peel *et al.* (2007) is a widely recognised (Spinoni *et al.*, 2015) and valuable tool for delineating between climate zones.

Maximising the number of studies accepted for meta-analysis is desirable for ensuring there is sufficient power to detect statistically significant differences among independent samples with a high degree of heterogeneity (Hedges and Pigott, 2001). As the data presented in Chapter 2 exhibits high variability and, in some cases, independent groups with $n < 5$ (figures 2.2-2.4), it is possible that efforts should be made to improve upon the methods used to aggregate data. However, as the findings from the meta-analysis were consistent with published primary literature, it can be argued that the study was useful for characterising the impacts of environmental change over varying degrees of aridity for both emerging ice-free arid ecosystems and shrub-encroached drylands.

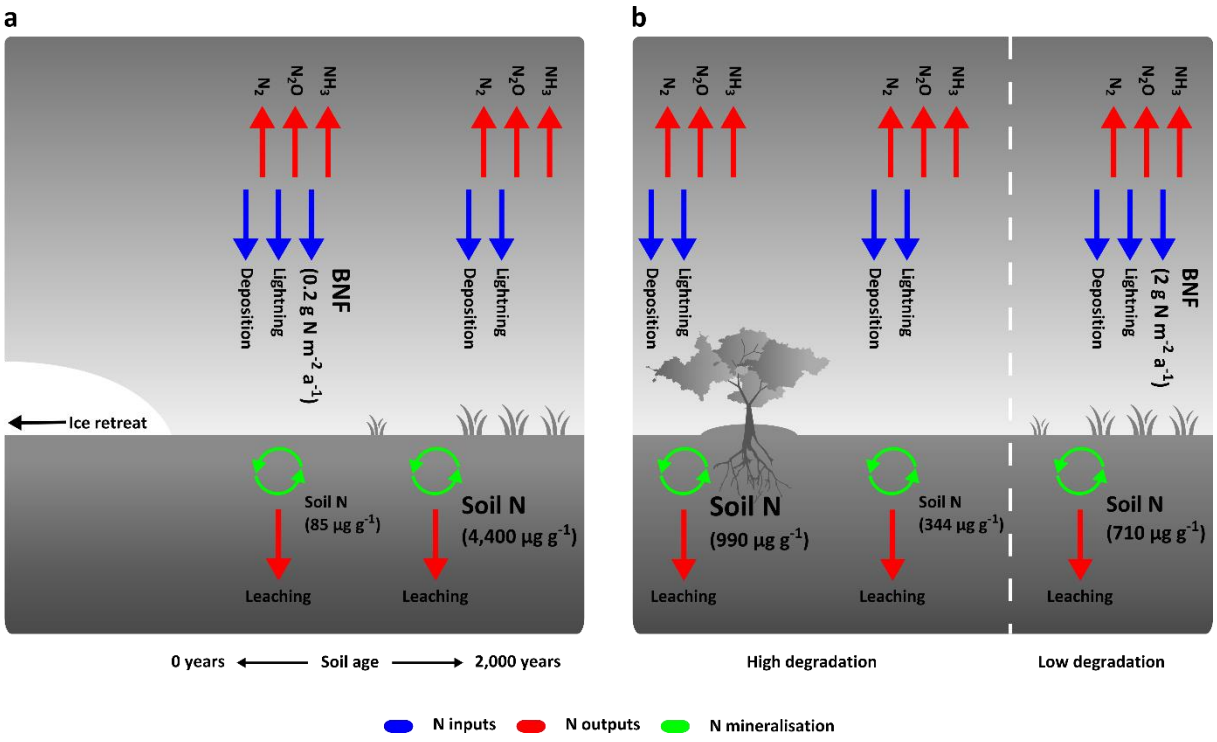

Figure 5.1 Conceptual model illustrating the inputs (wet and dry deposition, and fixation by lightning and biological processes), transformations (mineralisation) and outputs (emissions of dinitrogen (N_2), nitrous oxide (N_2O) and ammonia (NH_3)) of nitrogen (N) in expanding glacier forefields (a) and grasslands undergoing shrub encroachment (b), for which the distribution of the soil N pools, as well as inputs of N by biological N fixation (BNF) were quantified as part of this study. Absent N inputs were determined to be below the limits of detection.

5.4 Future work

The following recommendations for further research are based on the findings presented in this thesis:

- As discussed in section 3.5.2, the conversion from moles of ethylene (C_2H_4) produced to moles of dinitrogen (N_2) fixed during acetylene reduction assays should be calibrated with $^{15}N_2$. Whilst a widely cited stoichiometric conversion ratio of 3:1 (C_2H_4:N_2) was applied to data in chapters 3 and 4 of this thesis (Telling *et al.*, 2011); conversion factors for naturally-occurring cyanobacteria in soil have been shown to range from 1.9 to 6.1 (Belnap and Lange, 2001). Therefore, to improve the quantification accuracy of asymbiotic BNF rates in dryland soils, efforts should be made to calibrate acetylene reduction assay data using a $^{15}N_2$ tracer in accordance with methods outlined by Seitzinger and Garber (1987). This will be beneficial for advancing knowledge concerning the importance of asymbiotic BNF to arid ecosystems and allow direct comparisons to be made between potential rates of asymbiotic BNF in emerging ice-free arid ecosystems and shrub-encroached drylands.

- From the results in chapters 3 and 4, as well as the meta-analysis in Chapter 2, it was determined that >98 % of total N is typically contained within the organic phase of dryland soils. This is indicative of high immobilisation rates of mineralised N and suggests that soil organic matter content drives microbial activities in arid ecosystems (Vitousek and Howarth, 1991; Zeng *et al.*, 2015). As the availability of N is largely controlled by decomposition processes (Vitousek *et al.*, 2002b), incubation studies should be carried out in accordance with methods outlined by Zeng *et al.* (2015) to determine the distribution and extent of N mineralisation in surface soils between trajectories of succession and degradation, for which rates of mineralisation are calculated as the difference between final and initial concentrations of ammonium over time.
- Increasing atmospheric concentrations of radiatively-active gases are perturbing surface temperatures and precipitation patterns throughout the world (Trenberth *et al.*, 2007). As microbially-mediated processes, rates of asymbiotic BNF and N mineralisation are sensitive to changes in temperature and moisture availability (Li *et al.*, 2014; Rousk *et al.*, 2017). Thus, incubation studies should be conducted in accordance with the methods previously outlined in this thesis to assess the impacts of projected temperature change over the twenty-first century (e.g., Collins *et al.*, 2013) on the biological fixation and mineralisation of N by the soil microbial biomass along trajectories of succession and degradation in arid ecosystems. Incubation temperature regimes should be set according to the surface warming scenarios outlined by the Intergovernmental Panel for Climate Change (Stocker *et al.*, 2013).
- As discussed in sections 3.5.1 and 4.5.1, the soil organic C and total N contents of the forefield soils in Chapter 3 and grassland soils in Chapter 4 may have been underestimated by excluding the coarse fractions (>2,000 µm) from our analyses. Therefore, to understand the extent of the soil C and N pools in these systems, is it important that concentrations of C and N are determined for the coarse fractions of these soils using methods outlined by Cunliffe *et al*. (2016b).
- As discussed in section 3.5.1, the large unsampled interval between the 50- and 113-year-old glacier forefield soils makes it challenging to determine the rate at which the clay fraction, which increases rapidly between these years, accumulates. Therefore, it is recommended that soils between these ages (e.g., at 20-year intervals) are sampled for particle-size analysis.

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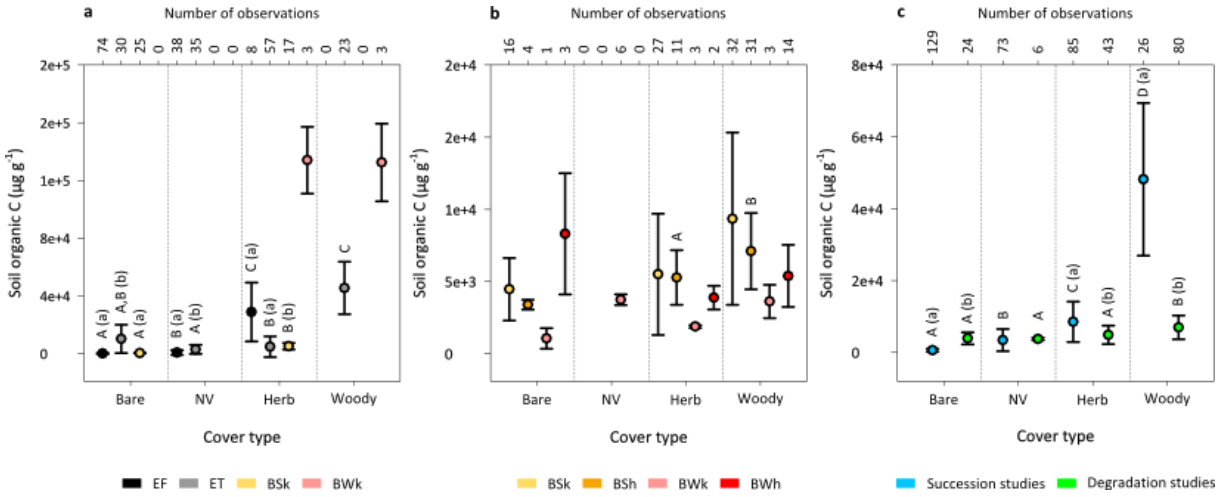
Appendices

Appendix A: Soil organic carbon in emerging and degrading drylands



Median soil organic carbon (C) concentrations identified by meta-analysis for surface soils (top ≤15 cm) under bare soil (bare), non-vascular (NV), herbaceous (herb) and woody cover types. Data, where present, is presented for emerging ice-free (succession; a) and shrub-encroached (degrading; b) arid ecosystems by the following Köppen-Geiger climate classes (Peel et al., 2007): polar frost (EF), polar tundra (ET), cold arid steppe (BSk), hot desert steppe (BSh), cold arid desert (BWk), and hot arid desert (BWh). The global median averages for these climate classes are also shown (c). See 'C:N' columns in tables 4.2 and 4.3 for source data. Error bars represent one median absolute deviation about the median. Upper- and lower-case letters indicate significant differences at $p < 0.05$ within classes by cover type and between classes within cover types, respectively (Mann Whitney *U* test for two groups; Dunn's test for more than two groups; groups with n < 5 were excluded from tests of significance).

Appendix B: Validation of acetylene reduction assay incubation procedure

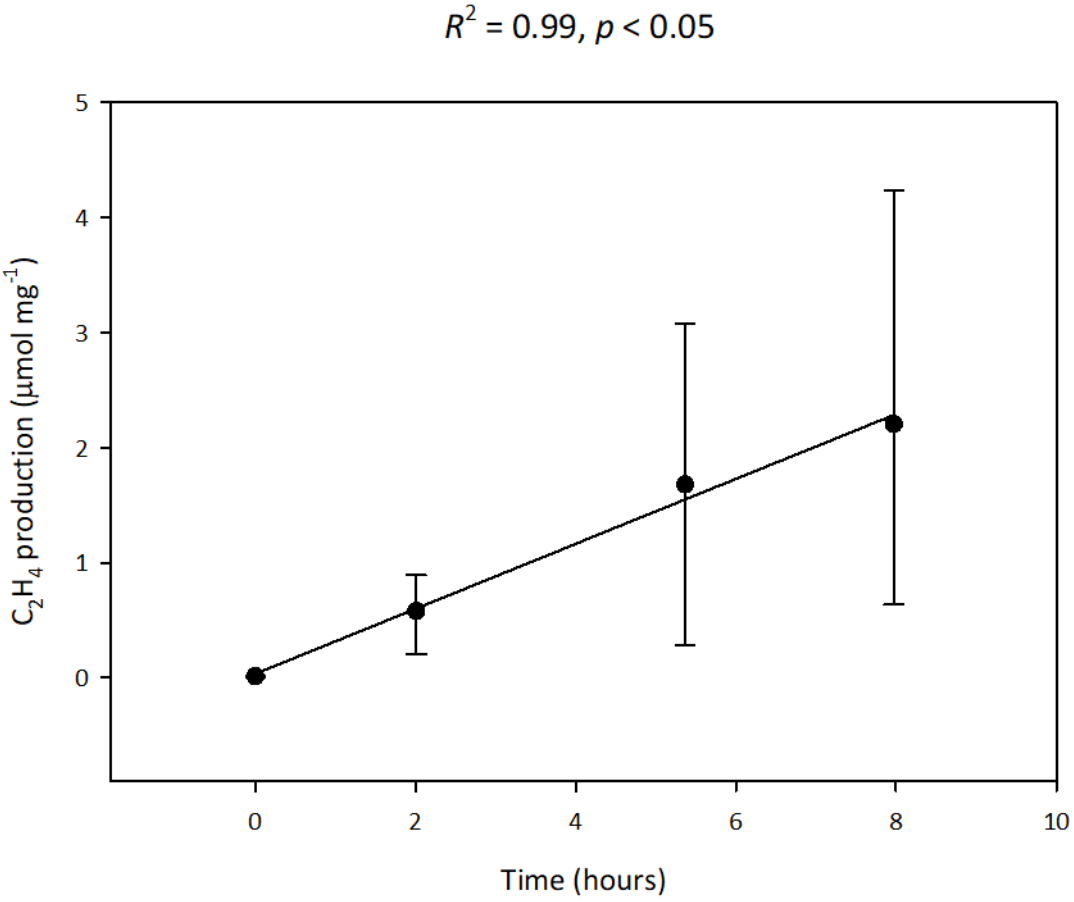
Rationale

Method validation is useful for evaluating if an analytical procedure is suitable for its intended use (Chan, 2011). As part of this research project, potential rates of asymbiotic biological nitrogen fixation (BNF) in dryland soils were assessed using the acetylene (C_2H_2) reduction assay technique, where the reduction of C_2H_2 to ethylene (C_2H_4) serves a proxy for nitrogen fixation (Belnap, 2001). However, as the capacity for asymbiotic BNF in these soils was unknown prior to undertaking this study, it was necessary to demonstrate that the methods outlined in section 3.3.6 of this thesis would not inhibit nitrogenase (enzyme which catalases the reduction of dinitrogen to ammonia) activity and were

capable of capturing the quantities of ethylene (C_2H_4) produced to avoid obtaining false-negative results. Thus, C_2H_2 reduction assays of nodulated white clover (*Trifolium repens* L.) root systems, which contain known nitrogen-fixing *Rhizobium* spp. (Francis and Alexander, 1972), were conducted to validate the appropriateness of the methods.

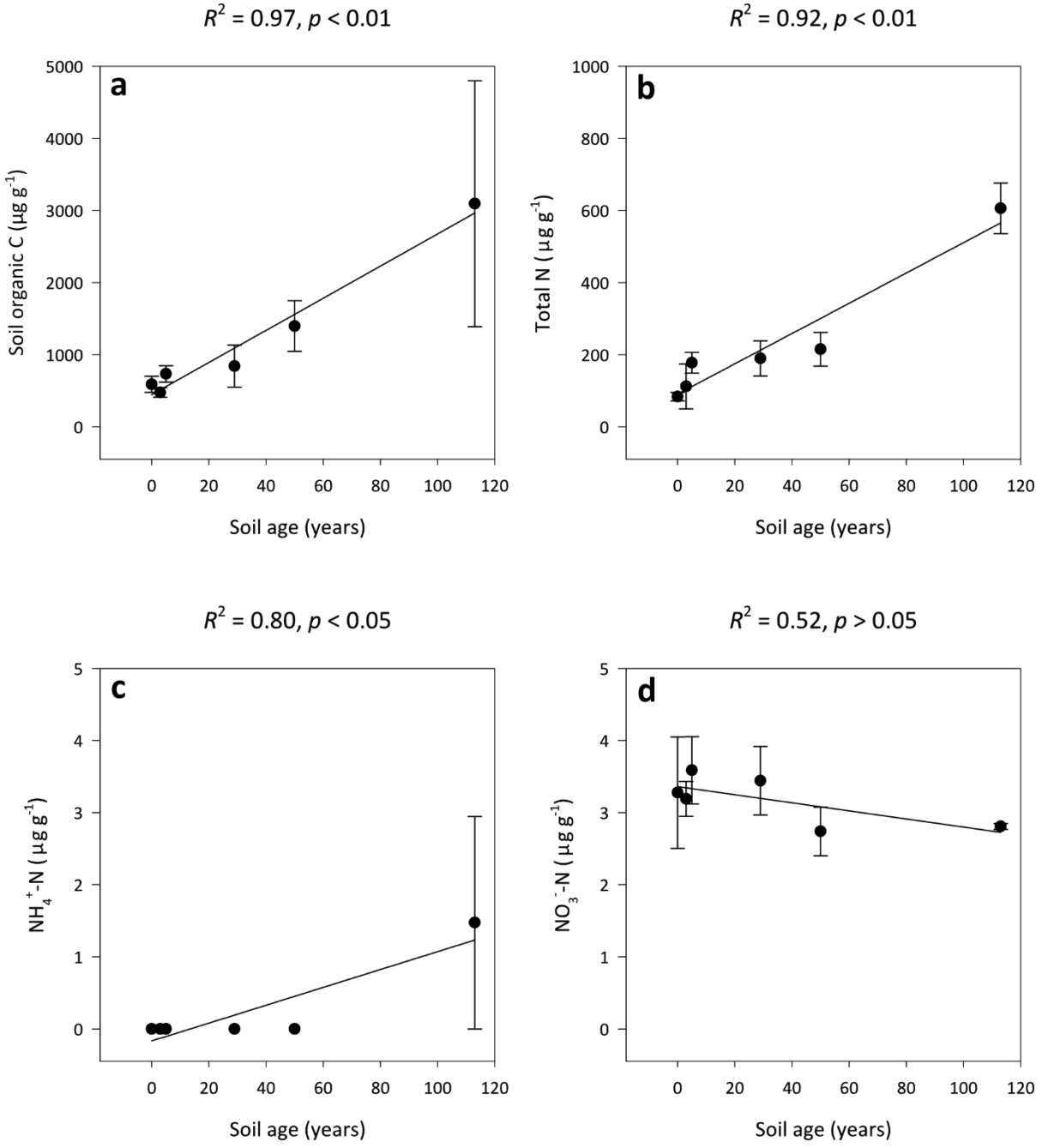
Materials and methods

Rates C_2H_2 reduction (i.e., C_2H_4 production) for nodulated roots systems of white clover were assessed using methods adapted from Telling *et al.* (2011). Three 60-day-old genetic clones of a mother clover plant, which had been grown in a glass house in accordance with methods described by Pears (2018), were carefully removed from their pots and placed in a bath of autoclaved Milli-Q water to wash all soil from their roots. Once the soil was removed, aliquots from the roots of each plant were blotted dry, separated from the shoots and transferred to 30 mL serum bottles and crimped shut using grey butyl stoppers. Subsequent to this, 10 % of the headspace from each serum bottle was replaced with 100 % C_2H_2 gas, which had been produced by adding Milli-Q water to technical grade calcium carbide (Sigma, St. Louis, MO, US). The samples were then incubated at 20 ±0.1 ˚C under 16 W florescent lamps (Sylvania, Garching, Germany) with emission spectra in the range of 300-700 nm and irradiance equal to 0.2 ±0.005 W m^{-2}. As rates of C_2H_4 production by disturbed legume-*Rhizobium* symbioses typically decline after a maximum period of eight hours (Sinclair, 1973), serum bottles were sampled at 0-, 2-, 5- and 8-hour intervals. The methods used to sample and analyse the headspace within each serum bottle are outlined in section 3.3.6 of this thesis. After the incubations were complete, nodules were immediately separated from the roots and weighed to determine the fresh nodule mass per serum bottle (mg). All sample measurements were blank-corrected using corresponding autoclaved root samples (126 ˚C for 30 minutes). The coefficient of variation for six 100 ppm C_2H_4 standards (BOC, Guildford, UK) was <5 %. The coefficient of variation for six 100 ppm C_2H_4 standards which had been stored in 3.7 mL Exetainers (Labco, Lampeter, UK) for a period of one month were also <5 %. Controls for natural C_2H_4 production in the absence of C_2H_2 and C_2H_4 consumption were consistently below the limits of detection (3.5 x10^{-6} µg mg^{-1}; signal-to-noise ratio of 3).



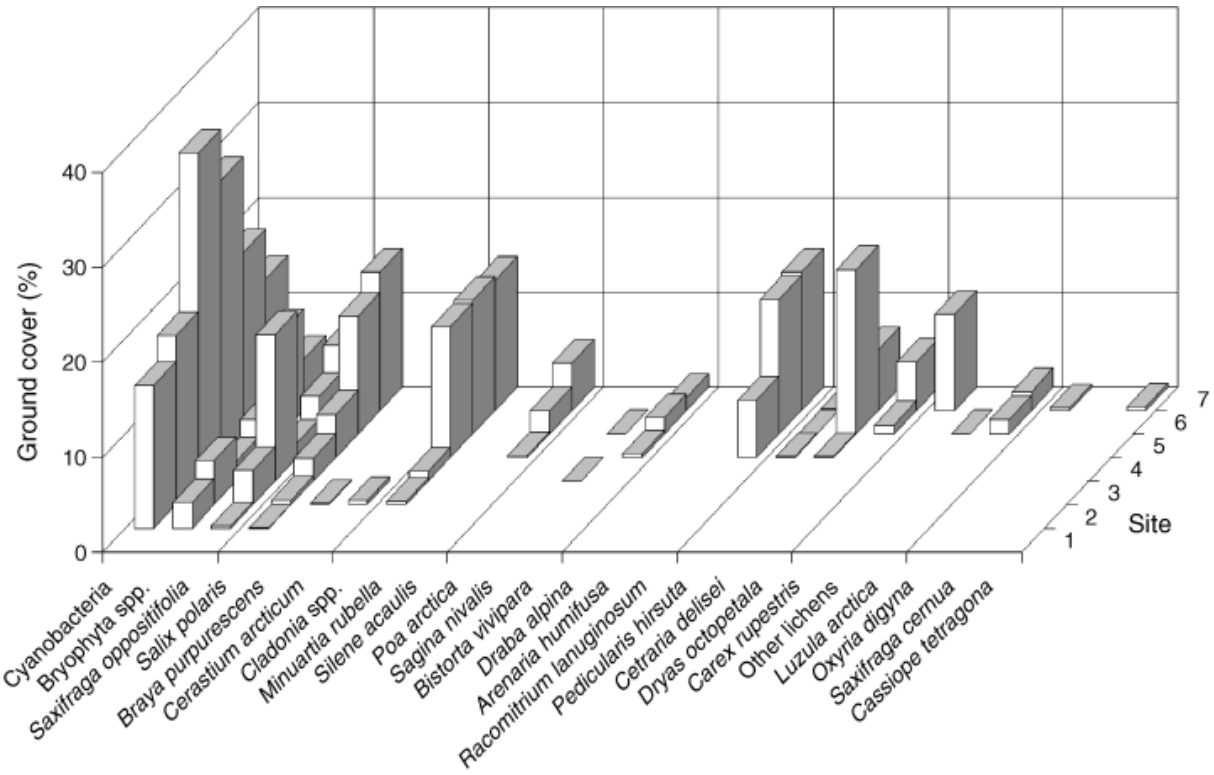
Median ethylene (C_2H_4) production by nodulated white clover (*Trifolium repens* L.) root systems, for which the linear production of C_2H_4 over time suggests that the incubation procedure does not inhibit nitrogenase activity. Results reported by nodule fresh mass. Error bars represent maximum and minimum values about the median (n = 3).

Appendix C: Concentrations of soil carbon and nitrogen in a glacier forefield



Median concentrations of soil organic carbon (C; a), total nitrogen (N; b), ammonium as N (NH_4^+-N; c) and nitrate as N (NO_3^--N; d) in the surface soil (top 10 cm) of the Midtre Lovénbreen glacier forefield, Svalbard, Norway (78˚55' N, 12˚10' E). Error bars represent one median absolute deviation about the median (n = 9 for soil <100 years old; n = 3 for soils >100 years old).

Appendix D: Vegetation cover by soil age in a glacier forefield



Percentage of vegetation cover by taxa within 0.5 m^2 quadrats in the Midtre Lovénbreen glacier forefield, Svalbard, Norway. Sites 1, 2, 3, 4, 5, 6 and 7 had been deglaciated for 2, 16, 37, 60, 100, 150, ~2,000 years, respectively (from Hodkinson *et al.*, 2003).

Appendix E: Images of Santa Rita Experimental Range sampling sites

Low ← Encroachment → High

1. S-4	2. S-28	3. S-44	4. S-55	5. S-56
Bare soil: 0 %	Bare soil: 2 %	Bare soil: 3 %	Bare soil: 6 %	Bare soil: 44 %
Grass: 96 %	Grass: 70 %	Grass: 53 %	Grass: 39 %	Grass: 0 %
Shrub: 4 %	Shrub: 28 %	Shrub: 44 %	Shrub: 55 %	Shrub: 56 %

Five sampling sites comprising a gradient of shrub encroachment in the Santa Rita Experimental Range, AZ, US (31˚54' N, 110˚53' W), where the percentage of grass cover decreases, and the percentage of bare soil and shrub cover increases between sites 1 and 5 (photographs by T. Turpin-Jelfs).